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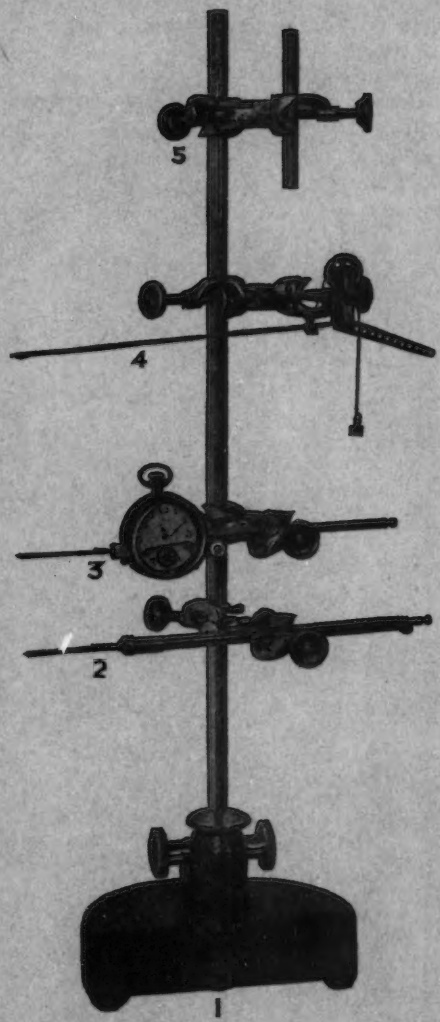

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COMPARATIVE PHYSIOLOGICAL VALUES OF FIVE CARBOHYDRATES, BASED ON GROWTH AND FECAL ANALYSIS

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Carbohydrates constitute not only the largest part of the food intake of the average person but are especially prescribed by the physicians in certain disease conditions where proteins and fats are contraindicated or where ease of digestion and assimilation is essential (1), (2). In a great many diseases where there is intestinal putrefaction as cause or complication, specific sugars are prescribed in an effort to promote the growth of an aciduric type of intestinal flora. Lactose and dextrine have proved most satisfactory, presumably due to the delay in absorption in the duodenum which leaves a significant residue to support the growth of the aciduric organisms in the colon. Rettger and Cheplin (3) have performed experiments on rats showing the relative efficiency of the different sugars in promoting this change in the flora, and have offered the explanation that there appears to be a definite relation between the rate of absorption or digestion, in the alimentary canal, of a utilizable carbohydrate and its tendency to effect a transformation in flora. Only those carbohydrates which reach the cecum and colon unchanged have a transforming influence.

As a consequence of this widespread use of carbohydrates as food and therapeutic agents it would be interesting to learn more concerning the relative physiological values of various types, correlating general condition over a long period of time, carbohydrate loss in the feces and intestinal flora conditions. A preliminary report by the author (4) showed striking variations in intestinal flora in rats on different types of cereal as the sole source of carbohydrates in the diet. The present paper presents the comparative results obtained by the use of different types of pure carbohydrates incorporated in the food mixture.

PROCEDURE. Rats of the same age and condition were fed on diets containing 60 per cent respectively of each of the following carbohydrates,

starch, dextrine, lactose, sucrose and maltose supplemented with protein; fat, minerals and vitamins to make the diet adequate as far as possible. The standard formula used throughout the experiment was carbohydrate 60, casein (Merck's) 18, salt mixture (Osborne and Mendel) 4, butter fat 18, with 0.4 gram of dry yeast daily. It is especially important that vitamin B be supplied in adequate quantity since Mattill (5) has shown that a vitamin B deficiency may interfere with the utilization of carbohydrate. In our own experience vitamin B deficiency has apparently delayed the growth of acidophilus. A form of roughage such as agar which would hasten motility and thereby favor the aciduric flora could not be used in these diets because the indigestible carbohydrate thus eliminated in the feces yielded reducing sugar on hydrolysis and thereby vitiated other results.

All of the rats on the experiment were observed carefully and weighed twice a week. In a few instances marked diarrhea occurred especially on the lactose diets and the feces were fluid instead of well formed as on the other diets.

Fresh fecal specimens were collected from the individual rats several times a month and complete bacteriological examinations were made by H. Tsuchiya following the careful technique described by him (6). These findings were entirely unprejudiced as no indication of dietary distinctions was made in the specimens submitted. Of the several criteria used in judging the flora, the percentage acidophilus was considered most significant and is used for comparison.

Feces for sugar analysis were collected for a period of a week and from two rats in a cage in most cases in order to obtain sufficient amount for quantitative study. Raised bottom cages were used which allowed the feces to drop onto a fine wire mesh with a blotter beneath to absorb the urine. Feces were collected daily and separated from the adhering particles of food. The small amount of fecal material which could not be recovered was estimated as nearly as possible and taken into account in the calculations. It has been noted by Roux and Goiffon (7) that the examination of stools from the human subject is not a reliable indication of digestion of carbohydrate since the motility and consequent chance for fermentation varies. Such a criticism has been overcome to some extent in these experiments by the fact that the diet is absolutely constant and the specimens collected for analysis were for weekly periods. The constipation and diarrhea noted in a few cases will be mentioned in the discussion of the results.

Sugar analyses were made on the hydrolyzed material as preliminary tests showed only traces or none when unhydrolyzed specimens were used. This in itself indicates that the sugar present had in most part escaped digestion, probably remaining unchanged and could not therefore be

absorbed. For our purpose the total carbohydrate present in the feces was the significant factor.

Feces were dried and ground and a weighed portion preferably five grams was added to 150 cc. of 2 per cent HCl. This mixture was autoclaved for 20 minutes at 15 pounds' pressure to effect a complete hydrolysis of the sugar. The suspension was neutralized with concentrated NaOH and made up to 200 cc. volume with distilled water and filtered. A number of preliminary attempts to titrate the reddish brown filtrate proved quite unsatisfactory due to difficulty in determining the end point in the Folin-McEllroy (8) quantitative sugar determination. Much more accurate results were obtained by decolorizing the solution with 10 grams of powdered charcoal and allowing it to stand 15 minutes before filtering. Uniform procedure was maintained in order to avoid possible error due to adsorption of sugar which however proved to be insignificant. Several control tests on solutions before and after using the charcoal showed no measurable amount of adsorption.

Discussion of results. Growth of rats on the starch, dextrine, maltose and sucrose diets was normal or above in every case and the males on dextrine and starch averaged considerable above the normal. On the other hand the maximum weight of the males on the lactose diet was 50 grams below the normal and the females somewhat below. The apparent reason for this retardation was the diarrhea which occurred more or less constantly in all rats on this diet. In the first group the diarrhea became so severe at one period that the rats were actually losing weight. A few grains of tanalbin were administered daily as an astringent to observe whether growth could be resumed without change in diet if the diarrhea were stopped. Diarrhea ceased in a few days and two weeks of the treatment were sufficient to stimulate increased rate of growth. However a marked drop in the percentage acidophilus from 80 and 95 to 15 and 13 occurred during the latter part of this period and for a few days following. A week later the high percentage of 80 and 85 per cent acidophilus had again been reached. This finding would indicate that a stagnation of residues in the colon even in the presence of lactose inhibits the growth of aciduric organisms. Other rats on the same lactose diet have shown varying degrees of diarrhea and corresponding retardation in growth.

The percentage of sugar lost in the feces even on the lactose diet was less than might have been suspected from the malnourished condition which resulted, but the relative amounts from the different types of sugars is rather significant, and fairly analogous to the average intestinal flora findings.

One must conclude from the findings that an aciduric type of intestinal flora does not in itself insure a good general condition of the animal over a long period of time if a high percentage of lactose or other slowly absorbed

carbohydrate has been used to affect the bacterial change. If the aciduric flora can be promoted by smaller amounts of lactose, growth might remain

TABLE 1

Report of fecal analysis

CARBOHYDRATE IN DIET	RAT NUMBER	TOTAL FECES PER RAT PER WEEK	PER CENT OF SUGAR IN FECES	FECAL SUGAR PER RAT PER WEEK	TOTAL FOOD CARBOHYDRATE	PER CENT FOOD SUGAR LOST	PER CENT ACIDOPHILUS	
							Rat	Average
					grams			
Starch	801	7.0	6.56	0.459	54.0	0.85		
60 per cent	801	4.7	9.26	0.435	54.0	0.80	801	2.2
	945,946	3.6	4.7	0.169	48.9	0.35	945	33.0
	945,946	4.2	5.2	0.218	44.4	0.50	946	38.0
	945,946	3.57	4.6	0.163	58.8	0.27		
			av. 6.06			av. 0.55		
Dextrine	807,808	11.7	13.33	2.226	57.0	3.9	807	34.0
60 per cent	807,808	8.5	8.7	0.739	53.4	1.4	808	41.0
	949,950	5.3	9.09	0.481	45.6	1.05	949	35.0
	949,950	5.15	8.0	0.412	49.8	0.83	950	56.0
	949,950	6.6	9.75	0.644	67.2	0.96		
			av. 9.77			av. 1.63		
Sucrose	809,810	7.9	7.4	0.585	54.6	1.07	809	3.0
60 per cent	809,810	4.9	7.84	0.381	42.6	0.89	810	2.7
	951,952	6.4	4.76	0.305	47.1	0.64	951	13.0
	951,952	3.42	3.65	0.124	47.4	0.26	952	12.5
	951,952	3.3	4.88	0.161	41.4	0.40		
			av. 5.71			av. 0.65		
Maltose	805,806	7.95	10.0	0.795	54.0	1.47	805	19.0
60 per cent	805,806	9.2	12.9	1.187	51.0	2.32	806	18.0
	947,948	5.0	6.45	0.323	39.6	0.81	947	31.0
	947,948	4.66	6.45	0.301	45.6	0.65	948	28.0
	947,948	2.2	11.11	0.244	39.6	0.62		
			av. 9.38			av. 1.17		
Lactose	803,804	6.15	50.7	3.118	52.8	5.9	803	87.0
60 per cent	803,804	10.6	27.05	2.231	51.0	4.37	804	83.0
	943,944	6.4	20.0	1.280	46.5	2.73	943	60.0
	943,944	9.1	20.0	1.820	66.0	2.75	944	66.5
	943,944	6.3	16.66	1.05	33.6	3.12		
			av. 26.88			av. 3.77		

normal. Report of such an experiment is made in the following paper on the use of different proportions of lactose in the diet.

Contrary to the results on lactose good growth was obtained on the 60 per cent starch, sucrose, maltose and dextrine diets where the percentage of acidophilus in the feces was low or medium. The table serves to show the marked correlation between carbohydrate residues and acidophilus content of the feces. This correlation is in close accord with that reported by Rettger and Cheplin on their short period experiments.

Wide variations in intestinal flora findings during the six months of the experiment may possibly be explained in one of two ways. The initial response of an acidophilus flora when the rats were first placed on the carbohydrate diets was much greater than after they had been on the diets for a month or two. In a second group tested the drop came even earlier and may have been hastened by the advent of very hot weather during the summer months, April to August. The previous group which showed less fluctuation and more prolonged response was conducted from January to June. This suggests a possible seasonal variation in intestinal flora or at least greater difficulty in promoting a change to the aciduric type in warm weather.

CONCLUSIONS

Intestinal flora findings show that the percentage of acidophilus runs parallel to the amounts of the sugar lost in the feces but in general the rate of growth is in the reverse ratio, i.e., lactose as the sole source of carbohydrate promotes the best growth of acidophilus but the poorest growth of the animal, while cornstarch commonly used in experimental feeding promotes excellent growth in the rat but affects no change in the intestinal flora.

High percentage acidophilus persists so long as diarrhea accompanies the feeding of lactose but intestinal stasis produced while on the same lactose diet allows the flora to revert to the putrefactive type.

By the use of certain carbohydrates the intestinal flora of rats may be changed to an aciduric type, but after continued use of the carbohydrate over a long period of time its effectiveness seems to diminish and the flora reverts toward the putrefactive type.

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COMPARATIVE PHYSIOLOGICAL VALUES OF DIFFERENT AMOUNTS OF LACTOSE, BASED ON GROWTH AND FECAL ANALYSIS

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In the preceding paper (1) 60 per cent of lactose as the sole source of carbohydrate in the diet of rats was found unfavorable for normal growth although the aciduric intestinal flora promoted thereby was supposedly favorable in character. Further work was therefore undertaken using various percentages of lactose combined with starch to ascertain if possible at what level of lactose intake, normal growth might be promoted, while still maintaining a predominance of B. acidophilus in the intestine.

Rats of the same age were placed on the diets given in table 1.

TABLE 1

	I	II	III	IV	V	VI
Lactose	60	45	30	15	5	
Starch		15	30	45	55	60
Casein	18	18	18	18	18	18
Butter fat	18	18	18	18	18	18
Salt mixture	4	4	4	4	4	4
0.4 gram yeast daily						

Corn starch was used as the supplementing carbohydrate because it is known to promote normal growth when used in "synthetic" diets but it has practically no influence on the intestinal flora. It may allow a putrefactive flora to predominate.

Intestinal flora examinations, fecal sugar analyses and growth records were made as in the previous experiment. There was a close correlation between the amount of lactose in the diet, the loss of sugar in the feces and the per cent of acidophilus in the intestinal flora. Growth was poor on the diets containing 60 and 45 per cent of lactose but practically normal on diets containing 30 per cent of lactose or less. B. acidophilus predominated in rats on diets I, II and III. It is therefore evident that at the

30 per cent level, lactose will permit normal growth and at the same time promote an aciduric type of intestinal flora.

There is nothing incompatible between normal growth and a moderately

TABLE 2

Report of fecal analysis

CARBOHYDRATE IN DIET	RAT NUMBER	TOTAL FECES PER RAT PER WEEK	PER CENT OF SUGAR IN FECES	FECAL SUGAR PER RAT PER WEEK	TOTAL FOOD CARBOHYDRATE	PER CENT FOOD SUGAR LOST	PER CENT ACIDOPHILUS	
							Rat	Average
					grams			
Lactose	983,984	8.8	15.38	1.353	54.0	2.5	983	77
60 per cent	983,984	9.77	18.18	1.776	51.0	3.48	984	79
	983,984	4.3	16.00	0.688	48.6	1.41		
	983,984	5.5	18.18	0.999	57.6	1.73		
			av. 16.93			av. 2.28		
Lactose	985,986	10.35	13.79	1.427	64.8	2.20	985	64
45 per cent	985,986	7.04	14.81	1.042	43.8	2.13	986	68
(starch)	985,986	6.1	13.8	0.901	45.0	2.0		
15 per cent			av. 14.1			av. 2.11		
Lactose	987,988	5.0	13.33	0.666	63.0	1.06	987	59
30 per cent	987,988	5.83	9.5	0.553	46.2	1.2	988	55
(starch)	987,988	5.7	7.84	0.446	55.2	0.81		
30 per cent			av. 10.32			av. 1.02		
Lactose	989,990	5.5	12.9	0.709	61.8	1.14	989	51
15 per cent	989,990	4.4	7.27	0.319	43.8	0.73	990	45
(starch)	989,990	5.0	7.4	0.370	49.2	0.75		
45 per cent			av. 9.19			av. 0.87		
Lactose	991,992	4.73	6.78	0.320	61.8	9.51	991	25
5 per cent	991,992	5.45	6.78	0.366	54.6	0.67	992	23
(starch)	991,992	5.25	7.14	0.371	52.5	0.7		
55 per cent			av. 6.9			av. 0.62		
Starch	993,994	4.62	4.77	0.220	49.8	0.44	993	14
60 per cent	993,994	3.57	7.27	0.259	53.4	0.48	994	17
			av. 6 02			av. 0.46		

aciduric intestinal flora but the means necessary for promoting an extreme change do interfere with growth in the young rat. These observations suggest that where a change in intestinal flora is desired it may be made

without interference in growth or well-being if lactose furnishes not more than 30 per cent of the diet or 50 per cent of the total carbohydrate.

The author wishes to express her appreciation to Dr. H. Tsuchiya for his untiring coöperation in making the bacteriological examinations of fecal specimens reported in both papers.

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NOTES ON BLOOD REACTIONS OF THE ALKALOIDS OF CEANOTHUS AMERICANUS

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The bark of the root of the shrub Ceanothus americanus has been shown by A. H. Clark (3) to contain certain alkaloids which according to J. T. Groot (8) have therapeutic value as an active hemostatic. This property has not been assigned to any specific clotting factors of the blood or the tissues. This study was therefore undertaken with a view to ascertain the location and the nature of the action of these alkaloids on the clotting mechanism.

Unfortunately for this purpose, the whole question of the clotting of shed blood is still *sub judica*. However, we may accept as demonstrated "that it is due immediately to a reaction between fibrinogen and thrombin, whereby fibrin is formed and deposited." We may, therefore, take as our point of departure the most generally accepted theory (9), (10), (11) that circulating blood contains all the necessary clotting factors, fibrinogen, prothrombin, and calcium but that these are prevented from reacting in the blood stream by the presence of antithrombin which holds the prothrombin in combination. In shed blood the restraining effect of the antithrombin is neutralized by the action of thromboplastin (tissue extract) derived both from the blood elements and from the external tissues. Howell (9) has shown that in the changes involved, thrombin may combine with fibrinogen, producing a clot, or with antithrombin preventing clotting. Antithrombin may combine with prothrombin or thrombin or it may combine with thromboplastin, thus releasing the prothrombin or thrombin. Fibrinogen may combine only with thrombin, and thromboplastin combines only with antithrombin. This interpretation has formed the basis for the following experiments which were devised to determine in what way these alkaloids might accelerate the clotting time of blood.

The drug used in these tests was supplied by G. C. Taylor, chief chemist of Flint Eaton Company. It was supplied in three states of purification, a semi-solid 7 per cent extract, the commercial preparation containing 1 mgm. of the alkaloid per cubic centimeter, and a purified solution containing 10 mgm. per cubic centimeter. For convenience the alkaloid will

hereafter be referred to as CA using the first letters of the generic and specific names of the plant from which they are extracted.

The first experiments were undertaken with a view to check up on the reported hemostatic properties of CA. Tests were made on guinea pigs, dogs, and man. The results were very similar throughout so there is no need of detailing more than the latter. For the guinea pigs and dogs, the purified alkaloids were administered subcutaneously, with the human subjects the commercial product was administered orally. Four fluid drams, being the normal dose given. A test of the clotting time was made immediately before administering the alkaloid CA and forty-five minutes afterwards. The clotting time for all experiments was determined by the exceedingly simple yet accurate method devised by Peterson and Mills (17), care being exercised to maintain a constant temperature and guard

TABLE 1

	CLOTTING TIME			CLOTTING TIME			CLOTTING TIME			CLOTTING TIME	
	Before	After		Before	After		Before	After		Before	After
1	6′10″	2′50″	11	8′05″	5′10″	21	3′15″	2′05″	31	5′35″	3′15″
2	1′35″	1′10″	12	6′15″	4′20″	22	4′	2′	32	4′15″	2′45″
3	6′	3′30″	13	5′	3′05″	23	6′15″	4′	33	3′50″	2′15″
4	4′10″	2′20″	14	6′15″	4′08″	24	6′40″	3′50″	34	6′	4′15″
5	4′	2′40″	15	5′10″	3′	25	4′05″	2′15″	35	4′25″	3′
6	3′20″	1′40″	16	6′05″	4′	26	7′10″	4′15″	36	4′30″	2′30″
7	4′	1′45″	17	6′40″	3′50″	27	3′20″	2′05″	37	5′15″	3′35″
8	5′05″	2′10″	18	2′15″	1′20″	28	5′15″	3′20″	38	2′45″	2′20″
9	7′	3′25″	19	6′45″	4′10″	29	2′40″	2′05″	39	4′30″	3′25″
10	11′05″	4′45″	20	5′15″	2′40″	30	5′25″	3′35″	40	4′50″	3′20″

against the effect of tissue juices as described by them. The human blood was obtained by venepuncture in the arm.

Average normal clotting time, 5 minutes .08 second
Average clotting time, 45 minutes after CA, 3 minutes .03 second
Average reduction in clotting time, 41 per cent

The above figures show that following the oral administration of CA, as above described, there is a marked reduction in the clotting time. It is remarkable that not one negative result was obtained. Following subcutaneous injection of the purified drug in guinea pigs and dogs, similar results were obtained.

Groot and Taylor have shown that a noticeable reduction in clotting time becomes apparent in from 10 minutes to 15 minutes and that it apparently reaches its maximum in from 45 minutes to one hour. These results have also been verified by my own tests.

Qualitative tests to show the relation between the clotting time of blood and the dosage of the commercial preparation of CA indicated that there is a gradual decrease in the clotting time varying with the increase in dosage up to six or eight fluid drams. Above that amount, the clotting time remains fairly constant. The maximum dose administered was twelve fluid drams. It would appear from these results that the reaction is quantitative in nature. Tests have shown that it does not induce intravenous clotting administered in any quantity.

An experiment was devised to test the action of CA on shed blood independent of body tissues. For this purpose a double cylinder syringe with a single needle was used. One drop of purified CA was placed in one of the cylinders. Five cubic centimeters of blood were drawn simultaneously into either cylinder. They were then gently agitated to insure thorough mixing. The clotting time of blood from each cylinder was determined. These were compared with clotting time on the same individ-

TABLE 2

NUMBER	WITHOUT TISSUE CONTACT		WITH TISSUE CONTACT	
	Without CA	With CA	Before CA	After CA
1	6′20″	5′35″	6′05″	3′40″
3	5′50″	5′40″	6′10″	3′55″
9	7′	5′50″	6′50″	4′25″
23	6′20″	5′05″	6′20″	4′05″
24	6′45″	5′15″	6′30″	3′25″
34	6′10″	4′55″	6′15″	4′15″

uals before and after CA administered orally. Individuals with comparatively long clotting time were selected for these tests. Nos. 1, 3, 9, 23, 24, 34 of the previous test were used.

The above results show that CA does accelerate the clotting of blood independent of tissue contact or digestion. Subcutaneous injection also bears out the latter point. Whether it undergoes any changes in digestion or in the blood stream is not known, or whether it is excreted from the body as such has not been tested. These results, however, would tend to indicate that it does not undergo an essential change in either process. The fact that the reduction in clotting time is not as marked as when given orally or subcutaneously might be explained either on the basis of reacting time or that it also plays a part in the tissue mechanism of clotting as well as that of the blood. The latter explanation is more probable and is apparently borne out by the fact that if tissue extract (thromboplastin) is also added, the clotting time is still further accelerated. (This experiment will be described later in this paper.)

To determine whether or not CA might be able to convert fibrinogen to fibrin, and therefore accelerate the process of clotting in the presence of thrombin, the following experiments were devised. Pure fibrinogen was prepared according to the method of Hammerstein. Pure thrombin was prepared according to the method of Howell (10). Dog blood was used in these tests.

Fibrinogen 0.5 cc., 1 drop purified CA, no clot
Fibrinogen 0.5 cc., 2 drops thrombin, clot 6 minutes
Fibrinogen 0.5 cc., 2 drops thrombin, 1 drop purified CA, clot 6 minutes

The above and a number of similar experiments make it apparent that CA does not cause the soluble fibrinogen to change into fibrin, thereby forming a clot. This same fibrinogen may readily clot by the addition of a small amount of thrombin but neither does the addition of CA with thrombin in any way change this action. It does not accelerate the action of thrombin in accomplishing this change.

It has been shown quite conclusively by Hammerstein that the rôle of calcium in clotting is in the conversion of prothrombin to thrombin. An experiment was therefore devised to test the possible influence of CA in this process. In oxalated human blood the prothrombin is not converted to thrombin because of the lack of free calcium ions. If a small amount of $CaCl_2$ is added to such oxalated blood plasma, a clot is formed, but if CA is added without calcium, it does not clot.

Oxalated human blood or plasma 1 cc., 1 to 5 drops purified CA, no clot
Oxalated human blood or plasma 1 cc., $CaCl_2$ (6 per cent) 2 drops, clot
Oxalated human plasma 1 cc., $CaCl_2$ (6 per cent) 2 drops, clot 5 minutes
Oxalated human plasma 1 cc., $CaCl_2$ (6 per cent) 2 drops, 1 drop CA, clot 5 minutes

It would therefore appear that CA plays no part in the conversion of prothrombin to thrombin, that it does not replace or accelerate the action of calcium in accomplishing this result.

Since both prothrombin and calcium salts are found in the blood stream, the reason why intravenous clotting does not normally occur, according to Howell (9), is because of the presence in the blood of an inhibitor (antithrombin) which holds the prothombin in combination preventing its action with calcium to form thrombin. In shed blood the antithrombin in turn is neutralized or caused to release the prothrombin by its union with thromboplastin released by the blood elements and the cells of the wounded tissue. Mammalian blood has been shown to differ from that of lower vertebrates in that in the latter, thromboplastin is not present (it is only found in the tissues, while in the former, it is found in both places).

Experiments were therefore performed to determine if CA might play a rôle in neutralizing the antithrombin or accelerating the thromboplastin

in accomplishing this result. Hen's blood was carefully drawn from the vessels into vaselined containers without contact with external tissues. It has been demonstrated that such blood contains antithrombin but that no thromboplastin is present. Controls set aside in the case of whole blood clotted only after several hours, while the centrifugalized plasma did not clot after two days. The addition of a small amount of tissue extract (thromboplastin) prepared according to the method of Cecil (1) produced a clot in similar specimens in a few minutes.

Hen's plasma 1 cc., 2 drops purified CA, no clot after 5 hours
CA was then added in varying amounts but no clot was obtained
Hen's plasma 1 cc., 5 drops thromboplastin, clot 7 minutes
Hen's plasma 1 cc., 5 drops thromboplastin, 1 drop CA, clot 4 minutes

The above and similar experiments demonstrates that in hen's blood CA alone does not neutralize the inhibiting qualities of antithrombin but that it does accelerate the action of thromboplastin in accomplishing this result.

It is a well-known fact that mammalian blood can be brought into an analogous condition to hen's blood by the simple method of peptonization. The peptonized plasma for these experiments was obtained from dog's blood as described by Cecil (1). That such peptonized plasma contains antithrombin has been demonstrated by Delezenne, Wolf and Morawitz.

Peptonized dog's plasma 1 cc., 2 drops purified CA, no clot
CA was then added in varying amounts but no clot was obtained
Peptonized dog's plasma 1 cc., 5 drops thromboplastin, clot 5 minutes
Peptonized dog's plasma 1cc., 5 drops thromboplastin, 2 drops CA, clot 3½ minutes

Experiments of the type of the above show that the action of CA on peptonized dog's plasma is the same as on normal hen's plasma extracted without tissue contact. Accepting the above conclusions of Wolf and others, the accelerative action of CA on clotting time must be due, not to a direct neutralization of the inhibiting action of antithrombin, but to the fact that, in some way, it aids the thromboplastin in accomplishing this result.

That the chemical constituents of the blood are not entirely passive in the reaction which causes the coagulation of blood is well established. In order to determine as to whether CA functions in a manner so as to influence blood constituents to bring about coagulation by either their quantitative suppression or augmentation and also whether such quantitative change, if found, would constitute a menace to the general welfare of the individual, a series of analyses of the blood of individuals was made before and after taking a normal dose of CA. The people who were chosen for this experiment were young men and women ranging from

twenty to thirty years of age, in perfect physical condition and not apparently deviating from the normal in any way.

The methods employed in the determination of the calcium in serum were those of Kramer and Tisdall (11) and that of G. W. Clark (2) and the method employed in the determination of the calcium in the whole blood was that of Luigi Condorelli (4). The method employed for the determination of magnesium in the whole blood was also that of Luigi Condorelli (5). Modifications of Kramer and Tisdall's method were employed in the determination of potassium and sodium (12). The well established methods of Folin-Wu were employed in the determination of sugar and nitrogen. In all of the methods, precaution was taken to eliminate errors. The ashing was carried on in carefully cleaned platinum crucibles. Carefully controlled heating prevented the volatilization of

TABLE 3

	BEFORE							AFTER						
	Whole				Plasma			Whole				Plasma		
	K	Na	Mg	Ca	Ca	Sugar	N_2	K	Na	Mg	Ca	Ca	Sugar	N_2
1	150	180	2.3	5.1	9.9	66	24.0	146	146	2.6	6.8	7.7	86	28.6
2	163	186	2.0	3.6	13.2	100	29.0	165	165	1.8	6.6	6.0	80	28.3
3	195	187	3.5	6.0	8.5	95	31.0	130	130	3.8	8.6	8.9	110	27.0
4	140	200	2.9	4.7	11.0	80	28.3	184	184	3.4	5.3	9.0	105	33.3
5	170	179	2.8	4.8	7.0	96	30.6	178	189	3.0	5.0	9.0	115	30.0
6	175	185	2.4	5.0	10.1	105	29.9	183	182	2.7	4.7	7.2	110	30.2
7	183	184	2.1	5.4	9.2	97	28.5	180	189	2.5	4.4	8.7	103	28.7
8	166	208	3.8	6.0	8.9	94	29.2	172	198	4.0	5.9	8.5	98	30.1
9	170	201	2.7	5.2	10.0	99	30.2	178	195	2.8	5.8	9.2	97	29.5
10	156	187	2.3	4.8	9.5	106	28.8	158	200	2.6	5.2	9.7	100	28.6

certain salts as well as the fusion of any of them. All reagents employed in this work were specially prepared and of greatest purity. Concentration of reagents received careful attention wherever precipitation problems were involved so as to obtain only the precipitation of the desired substance, and that quantitatively.

A comparison of the figures in the above tables brings out the fact that while changes in the concentration of the blood constituents are noted, these changes are not sufficiently great to cause a deviation in the concentration of the respective substances which could be construed as affecting the normal concentration for such substances in the blood of normal individuals. A slight rise in the sugar concentration of the blood after a dose of CA is due to the presence of ingredients in the commercial preparation of the compound which may temporarily in some instances of

glucose tolerance exhibit themselves in this manner. The lack of any appreciable fluctuation in the total non-protein nitrogen of the blood justifies the assumption that no change was affected in the concentration of urea, uric acid, or kreatine. The minor changes in the concentration of calcium, potassium, sodium, and magnesium seem to be of little consequence in relation to the problem under discussion.

The conclusions which may be drawn from the analytical data obtained is that the function of CA does not affect the concentration of the above constituents of the blood. The conclusion drawn from this fact is that CA functions by influencing the speed of one or more factors involved in coagulation. It is clearly to be seen that the speed of reaction is accelerated most easily by making available to the reaction as much of the reacting substance as possible and eliminating as much as possible of the products of the reaction.

Experimentally it has been shown that CA does not perform the functions of thromboplastin and it has also been indirectly demonstrated that the release of thromboplastin from the blood or tissue elements is not brought about by CA, for if such were the case, intravenous clotting might ensue, which is not the case. And, yet, it can be clearly discerned from the evidence on hand that CA does function in relation to thromboplastin. The natural conclusion to be drawn from all evidence is therefore to look upon CA as an accelerator of the action of thromboplastin.

The methods whereby it may accelerate the functions of thromboplastin may be divided into two main groups, direct chemical action, or catalytic action.

If CA accelerates thromboplastin by means of a direct chemical reaction, one may assume that thromboplastin is not released from the tissue or blood element as such, but rather in an unfinished form. The strong reducing power of CA may aid in the completion of the chemical unit of that substance which we describe as thromboplastin. Another view which may be taken is that thromboplastin is released from the blood or tissue element in combination with another chemical entity and that CA has a stronger affinity for said entity than thromboplastin. The result of such a fact would be merely the application of the old principles of mass action so well established by physical chemistry. The concentration of thromboplastin would increase very rapidly with resultant increase of rate of combination of thromboplastin and antithrombin which has a greater affinity for the same than it has for prothrombin. The increase of prothrombin would in turn cause, together with the aid of calcium, an increase in the thrombin available, to fibrinogen, to combine with, to form fibrin, to form the clot.

The other viewpoint which may be taken is that CA functions as a catalyst in either the chemical completion of the substance which has been

released from the blood element or tissue and which develops into thromboplastin or it may aid in the liberation of the same from any compound with which it may be combined.

A final view is that CA acts catalytically in the combination of antithrombin and thromboplastin. Such action would set the law of mass action to work by the elimination of the products of the reaction and permit more and more of the thromboplastin to be liberated.

Whatever the explanation may be of the ultimate chemical reaction, the facts as shown are of direct clinical interest in that they offer an explanation of the evidence that CA will reduce the clotting time somewhat if applied to wounded surfaces as well as if injected subcutaneously or administered orally, and further that it will not bring about intravenous clotting administered in any quantity.

SUMMARY

1. It has been shown that the alkaloids CA reduce the clotting time of blood and that this action may take place independent of body tissues or digestion as well as when administered orally or subcutaneously.

2. Experimental evidence seems to indicate that this action is due to an acceleration of the reaction of thromboplastin by the alkaloids CA.

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PANCREATIC DIABETES AND PREGNANCY[1]

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In 1914 Carlson and Ginsburg reported that the hyperglycemia subsequent to pancreatectomy was of slower onset, and did not become so marked in pregnant as compared with non-pregnant dogs. This seemed to confirm the conclusion drawn from previous observations that the internal secretion (of insulin) from the pancreas of the fetus may, in part at least, protect the mother against the consequences of pancreatectomy in the mother (Carlson and Drennan, 1911; Carlson, Orr and Jones, 1914). They stated that immediately after pancreatectomy the blood sugar rose to about 0.2 per cent, soon falling to 0.1 per cent, about which level it hovered until parturition when it rose again to 0.2 per cent. The findings in the urine paralleled those in the blood.

We have been unable to obtain evidence substantiating this view. When a completely depancreatized dog is kept on a constant diet of chopped meat, raw pancreas, and cane sugar, supplemented by injections of equal quantities of insulin twice daily, the phenomenon of oestrus supervenes about twice a year, when the animal can be impregnated. Markowitz and Simpson (1925) made observations on the sugar and nitrogen excretion of a dog that was impregnated eight months after pancreatectomy. At intervals during pregnancy total daily urine and feces were collected for periods of three or four consecutive days each. There was no change in the sugar excretion during the pregnancy. Twenty-four hours before parturition the fasting blood sugar was 0.429 per cent. Six pups were born, three being found dead.[2] One died shortly after birth. The remaining two pups suckled for one month, when they were weaned. The fact that the sugar balance did not change at any time during pregnancy

[1] The expenses of this investigation were met from a grant given by the Carnegie Corporation.

[2] It is of interest that about 36 hours after the pups were born, the animal developed hypoglycemia. This was explained as due to withdrawal of blood-sugar by the mammary gland for the synthesis of lactose. It was shown independently by Widmark and Carleus (1925) that the phenomenon of "milk fever" in cows following calving is due to hypoglycemia and that the favorite treatment of injecting air into the udder stops the secretion of milk, thus allowing the blood-sugar to return to its normal level.

would seem at first sight entirely to disprove the conclusion of Carlson and his co-workers. In the face of the large amount of insulin injected into the mother, it is possible however, according to the work of F. N. Allan (1924) that the fetal contribution of insulin would be overlooked.

TABLE 1

DATE	DEXTROSE	NITROGEN	BODY WEIGHT
	grams	*grams*	*kgm.*
March 27	3.63	14.0	
March 28	4.21	13.7	
March 29	1.75	14.1	
March 30	3.40	13.7	
April 9	4.28	13.45	8.0
April 10	3.34	13.4	
April 11	3.22	13.4	
April 12	2.50	13.4	
In view of the small excretion of sugar the dose of insulin was reduced to 10 units twice daily, the diet being unchanged			
April 20	5.83	14.1	8.5
April 21	7.43	13.45	
April 22	10.2	13.4	
April 23	12.3	13.95	
April 24	13.4	14.0	
April 30	9.00	13.3	9.1
May 1	8.75	12.6	
May 2	7.60	12.6	
May 3	13.6	12.1	
May 5	13.6	12.5	
May 6	11.8	12.4	
May 7	3.05	12.9	9.5
May 8	10.7	13.1	
May 9	5.20	12.3	
May 10	5.30	12.35	
May 11	5.54	11.9	
May 12*	12.0	12.5	
May 13	10.4	12.85	

* Blood sugar 5 hours after administration of food and insulin = 0.188 per cent.

In the present communication we are again reporting the sugar and nitrogen excretion of a depancreatized dog that developed oestrus and was impregnated. In addition we depancreatized two dogs that were in a state of advanced pregnancy. Blood and urinary sugar and urinary nitrogen were measured in the case of each dog. The respiratory exchange was also determined in a carefully controlled apparatus of the Benedict type.

RESULTS. *Pregnancy in a depancreatized animal.* Dog A, a small fox terrier weighing 6.8 kgm., was depancreatized on November 1, 1925. It was used for various experimental procedures involving occasional withdrawal of food and discontinuance of injections of insulin. On March 7, 1926, it was noted that the animal was menstruating. During the day it displayed the peculiar excitability of animals in oestrus and from March 10 to 13 it was kept in a room with male dogs. After this it was given twice daily a carefully weighed diet of 200 grams chopped meat, 50 grams raw pancreas, 40 grams sucrose supplemented by 12 units of insulin. The food and insulin were administered at 10:30 a.m. and 8 p.m. The same insulin was used during the entire experiment. On March 26 at 10 a.m. the animal was catheterized and the urine discarded. It was then placed in a separate metabolism cage. In table 1 are shown the daily excretion of nitrogen and glucose in the urine.

May 13, 8:30 p.m. Animal in labor; one pup born alive and vigorous. Mother given 10 units insulin and food which it refused. There were no symptoms of hypoglycemia.

12 o'clock midnight. Three vigorous pups were born.

May 14. One of the pups found dead the remaining two being alive and vigorous. Animal took a lively interest in the pups and objected to anyone handling them. Although pups were suckling vigorously no milk was secreted, the mammary glands being scarcely enlarged and devoid of milk on expression.

12 o'clock noon. Blood sugar = 0.319 per cent. Given 6 units insulin. Refused her food which was accordingly left in the cage.

10 p.m. Had eaten half of her food. Another pup dead, the remaining one was suckling vigorously and possibly getting some milk since the posterior four mammary glands had become somewhat enlarged. Dog was given 5 units insulin and half its usual supper.

May 15. Third pup dead. Dog had eaten about half its meal. Given 10 units insulin and full meal.

May 16–20. Appetite poor. Animal took only a little of its food at a time.

May 26. Appetite normal. Catheterized. Given its full rations of food and 10 units insulin. Sugar and nitrogen excretion shown in table 2.

Pancreatectomy in Pregnant Animals. Dog B, small fox terrier.

June 16, 4 p.m. During laparotomy for the purpose of making a reverse Eck fistula the dog was found to be in a state of advanced pregnancy so that the plan was changed and pancreatectomy with perinostomy was quickly performed. The ether anesthesia lasted for about half an hour and an excellent recovery was made.

June 17, 10:15 a.m. Animal in excellent condition. Blood sugar = 0.222 per cent.

11:07 a.m. Three units insulin injected.

2:20 p.m. Blood sugar = 0.090 per cent.

6:15 p.m. Blood sugar = 0.140 per cent. Three units insulin injected. It was offered 100 grams meat plus 25 grams raw pancreas which it refused.

June 18, 12:20 p.m. Blood sugar 0.176 per cent. Three units insulin injected; 100 grams meat, plus 25 grams pancreas were left in its cage.

5:25 p.m. Had eaten about half the food; two units insulin injected.

June 19, 5:25 p.m. Blood sugar = 0.252 per cent, 3 units insulin injected. Given 100 grams meat plus 25 grams raw pancreas, of which it ate about a half.

June 20, 10:35 a.m. Remainder of food has been eaten. The bladder was emptied by catheter and the dog was placed in a separate metabolism cage.

June 21. Placed in respiratory cabinet with the results shown in table 3.

12:10 p.m. Blood sugar = 0.269 per cent.

1 p.m. Catheterized. Total nitrogen = 10.3 grams. Dextrose = 5.0 grams. D/N = 0.48. Three units insulin injected.

2:10 p.m. Given 100 grams meat plus 25 grams pancreas.
2:20 p.m. Animal in labor. Gave birth to one pup, alive.
3:10 p.m. Another pup born, alive.
10:15 p.m. Two units insulin injected.
June 22, 8 a.m. Found 2 more pups born, alive.
12 noon. Offered 125 grams meat, 25 grams pancreas which it refused.
4:30 p.m. Blood sugar 0.326 per cent. Slight amount of milk observed in mammae. Animal showed no interest in her pups, two of which died during the day. The remaining two were vigorous. Three units insulin were injected.

TABLE 2

DATE	DEXTROSE	NITROGEN	BODY WEIGHT
	grams	*grams*	*kgm.*
June 10	8.7	14.1	8.6
June 11	9.4	13.9	
June 12	11.6	13.9	
June 13	11.0	14.0	

TABLE 3

TIME	O_2 CORRECTED	CO_2 WEIGHT	CO_2 VOLUME	R.Q.
	cc.	*grams*	*cc.*	
10–11 a.m.	4608	6.055	3082	0.669
11–12 a.m.	4833	6.62	3370	0.697

TABLE 4

TIME	O_2 CORRECTED	CO_2 WEIGHT	CO_2 VOLUME	R.Q.
	cc.	*grams*	*cc.*	
10:15 a.m.–11:15 a.m.	4137	5.745	2924	0.707
11:15 a.m.–12:15 p.m.	3575	5.35	2723	0.762
12:20 p.m. Given 20 grams glucose in 100 cc. saline by stomach tube				
12:50 p.m.–1:50 p.m.	3650	4.77	2428	0.665
1:50 p.m.–2:50 p.m.	3719	5.33	2713	0.729
2:50 p.m.–3:50 p.m.	3471	4.86	2474	0.713
3:50 p.m.–4:50 p.m.	3645	5.25	2672	0.733

Average R. Q. before glucose = 0.732.
Average R. Q. after glucose = 0.710.

June 23, 11 a.m. Four units insulin injected and 100 grams meat, 25 grams pancreas, plus 5 grams sucrose given. Slightly over half of this was eaten.
5 p.m. Remaining two pups died, probably of inanition.
June 24, 12:45 p.m. Blood sugar 0.338 per cent.

Dog C, small fox terrier.
June 16, 4:30 p.m. During laparotomy for reverse Eck fistula the dog was found to be in a state of advanced pregnancy. Pancreatectomy with perinostomy was performed. Anesthesia for half an hour. Excellent recovery.

June 17, 10:30 a.m. Blood sugar 0.228 per cent.

11:40 p.m. Three units insulin injected.

2:42 p.m. Blood sugar 0.114 per cent.

6:30 p.m. Blood sugar 0.100 per cent. Three units insulin injected. Ate 100 grams meat and 25 grams raw pancreas.

June 18, 12:35 p.m. Blood sugar 0.287 per cent; three units insulin injected; 100 grams meat plus 25 grams pancreas given. Ate only a little of this.

5:25 p.m. Three inits insulin injected. Remainder of previous meal removed, had eaten less than half.

June 19, 5:35 p.m. Blood sugar 0.253 per cent; three units insulin injected; 100 grams meat plus 25 grams pancreas given. Appetite improved but did not eat more than half.

June 20, 10:30 a.m. Has eaten remainder of yesterdays meal. Urinated; sample discarded. Placed in a separate metabolism cage.

June 21, 12:05 p.m. Blood sugar 0.248 per cent.

1 p.m. Catheterized. Total nitrogen = 6.06 grams. Dextrose = 9.24 grams. D/N = 1.52. Three units insulin injected.

2:16 p.m. 100 grams meat plus 25 grams pancreas given.

10:15 p.m. Three units insulin injected.

June 22, 12 o'clock noon. One-hundred and twenty-five grams meat plus 25 grams pancreas given, ate about half.

June 23, 11 a.m. Four units insulin injected. Given 100 grams meat, 25 grams pancreas plus 5 grams sucrose.

5 p m. Has eaten a little less than half of its food.

June 24, 9:40 a.m. Put into respiratory cabinet with the results shown in table 4. The animal behaved very well while in the respiratory cabinet, being asleep most of the time.

5 p.m. Four units insulin injected. Given 100 grams meat, 25 grams pancreas plus 5 grams sucrose.

June 25, 9 a.m. Gave birth to 2 pups during the night. Both alive and vigorous. Animal is very attentive to them.

June 26, 11:30 a.m. Blood sugar 0.248 per cent; 3 units insulin injected; 125 grams meat, 25 grams pancreas plus 5 grams sucrose given. Eaten with relish.

Pups quite vigorous and mother paying considerable attention to them. Apparently no milk in mammae.

June 27. Both pups much weaker, getting no milk. Chloroformed.

DISCUSSION AND SUMMARY. Dog A was impregnated several months after pancreatectomy and its metabolic balance was determined during pregnancy. It went to full term, 3 vigorous pups being born on May 13. There was no change in the excretion of sugar except on May 7, 9, 10 and 11, on which days the glycosuria was diminished. The decrease however was not greater than is consistent with biological variation. The absence of hypoglycemia following parturition is probably dependent on the fact that no milk was secreted by the mammary glands.

Dogs B and C which were in a state of advanced pregnancy, were quickly depancreatised. Both animals went to term, healthy pups being born. In spite of frequent injections of insulin, typical diabetes supervened, as indicated by hyperglycemia, glycosuria and the respiratory metabolism.

In dog B the R. Q. was "diabetic," i.e., about 0.69. In dog C, owing no doubt to the effect of recently injected insulin, the R. Q. was 0.73, falling after the ingestion of 20 grams glucose to 0.71.

Although in both dogs B and C the blood sugar on the day following pancreatectomy and before any insulin had been injected, was somewhat less than that usually observed at this time in non-pregnant animals it is quite clear from the further history of the animals and the respiratory quotients that the subsequent diabetic symptoms were as severe as would be expected in consideration of the insulin injections. These were given so that the animal might be carried to full term to see whether hypoglycemia consequent upon the secretion of milk would supervene following parturition. In this regard we were disappointed since the mammary glands failed in both animals, as also in animal A, to become adequately developed to furnish any milk.

Accordingly we cannot find in our experiments any confirmation of the results reported from Carlson's laboratory.

CONCLUSIONS

1. The carbohydrate balance remained unchanged from day to day during pregnancy in a depancreatised dog treated with insulin.

2. In two dogs which were depancreatised during the later stages of pregnancy and given small doses of insulin the diabetic symptoms as judged from the behavior of the blood sugar, the D:N ratio and the R. Q. were as severe as would be expected after pancreatectomy in non-pregnant animals.

3. There is therefore no evidence that the fetal pancreas can secrete into the maternal organism a sufficient amount of insulin to offset the diabetic condition caused by pancreatectomy.

4. The mammary glands in all three animals failed to hypertrophy properly.

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THE INTRAVENOUS ADMINISTRATION OF AMINO ACIDS TO DECEREBRATE AND URETHANIZED CATS

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The specific dynamic action of foodstuffs has been widely investigated, especially by Lusk, who has so amply reviewed the literature that it needs no full summary here. Since most of the work has been upon unanesthetized animals fed by mouth, results have been secured only at the expense of much time and care in training the animals. Moreover, this method allows no complicating experimental procedure during observations upon the metabolism, and long periods must be run, during which the rate of absorption, the blood changes and other variables cannot be investigated. Such limitations could be partly removed by using anesthetized (or decerebrate) animals, and this might permit further studies of the mechanism of the specific dynamic action. The experiments reported here demonstrate such a method.

PREVIOUS EXPERIMENTS. Von Mering and Zuntz (1) in 1877 injected peptone solutions intravenously and obtained a rise in metabolism which was slight in comparison with that secured after administration *per os;* but since no record of muscular activity was taken, and since peptones are not easily broken down to amino acids in the blood, this work was of little real value. Tangl (2) injected 5 per cent NaCl intravenously into nephrectomized animals and secured an increase in heat production of 15 to 35 per cent; a result not observed after oral administration (3); whereas injection of 5 per cent urea on two separate occasions caused rises of 2.8 per cent and 27 per cent. In 1914 Heli and Wolfe (4) observed specific dynamic action following intravenous injection of glycocoll into dogs decerebrated by Langley's starch method, but their results are open to criticism, for an examination of their data reveals variations in the control periods and temperature of their animals which are not considered in calculating increases in metabolism. The blood pressure readings were often omitted or were, in certain cases, below 80 mm. Hg, which Aub (5) has since shown to be a critical level in the maintenance of metabolism. Their single control experiment shows greater variations in oxygen consumption than those which they consider to demonstrate a specific dynamic action.

Krzywanek (6) rapidly injected into the jugular vein of unanesthetized dogs, solutions of glycocoll and alanin, and after each observed considerable rise above the basal metabolism which began almost immediately after injection and persisted for 3 to 4 hours.

METHOD. Our experiments were performed on anesthetized cats in two series. In the first, urethane or paraldehyde was the anesthetic—8 cc. 25 per cent aqueous solution of ethyl urethane or 1.7 cc. paraldehyde per kilo weight by stomach tube; in the second, anesthesia was obtained by decerebration.

The animals received nothing but water for 18 to 24 hours before the experiment. Immediately after anesthetization the animal was placed on an electric heating pad, and a rectal thermometer was inserted. The left carotid artery was connected with a citrate-mercury manometer for blood pressure readings. Intravenous injections, after basal metabolism samples were taken, were made through a cannula tied in the left femoral vein. Before determination of the basal metabolism and preceding every period throughout the experiment, the heart rate, respiratory rate, blood pressure and temperature were recorded. The metabolism was determined by a method already described (5), Tissot valves attached directly to a Y-shaped tracheal cannula delivered the expired air into 8 liter spirometers. No metabolic observations were considered valid if the blood pressure fell below 80 mm. Hg, if any spontaneous movements or rigidity occurred, or if temperature readings varied more than $\pm 0.5°C$. After each experiment an autopsy was performed to prove the absence of food in the digestive tract.

The respiratory gases were collected for periods of 8 to 10 minutes. Three separate determinations of the basal metabolism were obtained in all experiments and were followed by repeated observations after the intravenous injections were made. The gases collected during each period were mixed, the volume determined, and a sample analyzed in a Haldane gas apparatus. Checks of the apparatus were made with room air before analysis of experimental samples. The results recorded are averages of two or three analyses of the same samples reduced to standard conditions of pressure, temperature and humidity.

Five grams of the injected substances were dissolved in 50 cc. mammalian Ringer solution (except glutamic acid which was dissolved in 5 to 10 cc. Na_2CO_3 solution and then made up to 50 cc. with mammalian Ringer and brought to a pH of 7.4). They were injected intravenously at rates varying from 1 to 7 cc. per minute in different experiments.

Series I: Anesthetized animals. Four experiments were performed with urethane (tables 1 and 5). The basal metabolism remains constant for at least four and a half hours with this anesthetic. In experiments III, IV and IX, 5 grams of glycocoll were injected; in experiment VIII, 10

grams. It will be observed that all increases of metabolism above the basal level were slight. In fact, doubling the dose of amino acid in experiment VIII not only did not elicit a specific dynamic action, but resulted in a variation of −3.1 per cent below the basal level. When paraldehyde was substituted for urethane there was no more pronounced effect. With both anesthetics a rise in the respiratory quotient followed injection of glycocoll and persisted to the end, except in experiment IX. Table 1 demonstrates that this is not caused by increased elimination of CO_2 following an increased minute volume of respiration.

Series II: Decerebrate animals. Because no specific dynamic action occurred during narcosis, it was suspected that drugs might in some way suppress the mechanism which stimulated metabolism, and it therefore

TABLE 1

Effect of glycocoll, injected intravenously, on the metabolism of narcotized cats

EXPERIMENT NUMBER	ANESTHETIC	AVERAGE CALORIE RISE AFTER INJECTION		AVERAGE RESPIRATORY QUOTIENT		AVERAGE RESPIRATORY VOLUME	
				Before injection	After injection	Before injection	After injection
		per cent	*calorie per hour*			*cc. per minute*	*cc. per minute*
III	Urethane	+10.4	+0.58	0.76	0.86	448	489
IV	Urethane	+9.7	+0.59	0.73	0.87	502	575
VIII	Urethane	−3.1	−0.18	0.69	0.83	1677	683
IX	Urethane	−4.5	−0.23	0.76	0.74	552	402
XI	Paraldehyde	+10.7	+0.65	0.81	0.91	1140	1142
XII	Paraldehyde	+6.0	+0.24	0.72	0.83	688	513

appeared desirable to secure anesthesia without drugs. This was accomplished by decerebration. The animal was etherized, a tracheal cannula rapidly inserted and both carotid arteries tied. The cat was then placed on the Sherrington decerebrator with its head firmly secured in the proper position. A cut was made 2 mm. proximal to the midpoint of the external occipital protuberance through the cerebrum to its ventral surface. In some cases section was made more anteriorly to avoid too much interference with the brain stem, and as a rule it was well forward of the tentorium and through the upper part of the mid-brain or posterior part of the thalamus. During section the vertebral arteries were compressed to reduce hemorrhage. In no case was more than 10 cc. of blood lost, and since metabolism is not appreciably affected until 2 per cent of the body weight (60 cc. for a 3-kilo cat) is lost, such slight hemorrhage was disregarded in the results (5), (7). After the section, the occipital lobes and cerebrum forward of the cut were mutilated. The shock and arterial compression momentarily hindered respiration, but this was generally

readily restored upon relieving the medullary ischemia. Approximately one-third of the cats remained in a satisfactory condition. Since gas samples were not taken for an hour or more after etherization, the presence of ether in the collected respiratory gases could be excluded. In a few cases anesthesia was facilitated by cerebral pressure produced by trephining both sides of the skull over the motor cortex and inserting under the skull cap small pads of absorbent cotton (8).

TABLE 2

Effect of glycocoll, injected intravenously, on the metabolism of decerebrate cats

EXPERIMENT NUMBER	SOLUTION INJECTED	AVERAGE CALORIE RISE AFTER INJECTION		AVERAGE RESPIRATORY QUOTIENT		AVERAGE RESPIRATORY VOLUME	
				Before injection	After injection	Before injection	After injection
		per cent	*calories per hour*			*cc. per minute*	*cc. per minute*
XL	50 cc. mammalian Ringer's	+3.2	0.20	0.69	0.80	626	666
XXVII	50 cc. 10 per cent urea	+7.9	0.57	0.76	0.78	565	1444
XV	5 grams glycocoll	+23.6	2.78	0.72	0.81	909	1358
XVI	5 grams glycocoll	+26.6	1.82	0.80	0.82	1358	3110
XXIII	5 grams glycocoll	+49.0	3.66	0.73	0.85	753	3179
XLII	5 grams glycocoll	+19.6	1.33	0.79	0.85	1196	1386

TABLE 3

Effect of glutamic acid, injected intravenously, on the metabolism of decerebrate cats

EXPERIMENT NUMBER	AVERAGE CALORIE RISE AFTER INJECTION		AVERAGE RESPIRATORY QUOTIENT		AVERAGE RESPIRATORY VOLUME	
			Before injection	After injection	Before injection	After injection
	per cent	*calories per hour*			*cc. per minute*	*cc. per minute*
XXX	+49.0	4.90	0.62	0.72	370	946
XLI	+1.2	0.26	0.73	0.64	869	808
XXXII	+6.6	0.49	0.77	0.68	544	506
XLIII	+1.9	0 12	0.80	0.84	1517	1957

With these decerebrate preparations two experiments were run as controls to eliminate the possibility of changes in metabolism following intravenous injections (table 4). In experiment LX, 50 cc. of mammalian Ringer solution were injected. The increase in metabolism is well within the limits of experimental error. In experiment XXVII, similar results were obtained when 50 cc. of a 10 per cent urea solution were injected. This can be regarded as a control observation since injected urea apparently undergoes no chemical changes in the body. These

findings are in harmony with those of Raeder (9), Henriques (10) and Krzywanek (6).

a. Glycocoll. Four experiments with 5 gram doses of glycocoll gave very decided increases in metabolism, as shown in tables 2 and 4. The average increase in the four experiments was 29.9 per cent for cats with an average weight of 3.0 kilograms. But this specific dynamic action was only observed for one to two hours after injection because of the death or collapse of the animal. The rise in metabolism appears to occur almost immediately on the introduction of the amino acid into the blood,—in experiment XV a rise of 21.7 per cent was observed nine minutes after the beginning of the injection.

It is pertinent to question whether the increased metabolism may not be due to increased activity of the respiratory muscles, for the respiratory volume increases noticeably after the injection of glycocoll. That this is not the case is evident from the lack of correspondence between respiratory volume and rise in metabolism. Thus in experiment XL, the respiratory volume per minute was more than doubled while the rise in metabolism was only 7.7 per cent, and in experiment XV the respiratory volume increased about 40 per cent and the calorific output 23.6 per cent. To eliminate any possible doubt, experiment XLII was performed under artificial respiration.[1] With the respiratory volume thus made constant, a definite specific dynamic action was observed, in spite of the loss of the sample which probably represented the height of reaction.

A rise in the respiratory quotient appears in these experiments simultaneously with the increase in metabolism. This rise was also observed by Krzywanek who suggested that it might be due to combustion of carbohydrate radicles derived from the injected amino acids. According to Abelin (11) and others, however, it is caused by the increased glycogenolysis in the liver, produced by stimulation of the sympathetic nervous system similar to the effect of adrenalin. Whatever the mechanism of this rise in the respiratory quotient, it seems independent of the phenomenon of specific dynamic action, for the increased respiratory quotient may be observed in urethanized cats when a specific dynamic action does not follow injection of glycocoll. In no case was the total caloric rise after injection more than 50 per cent of the physiological heat of combustion of the glycocoll introduced. The average total caloric rise was 2.72 calories for 5 grams of glycocoll which is comparable to Lusk's value of 3.7 calories for 5.5 grams of glycocoll per os. The correct figures are

[1] Normal respiration was stopped by increasing the intracerebral pressure with cotton plugs. Artificial respiration was then given by Drinker's apparatus, which delivers a constant volume of air at constant rate with constant pressure, in this experiment about 1200 cc. per minute. We wish to take this opportunity to thank Dr. C. K. Drinker for the use of this apparatus.

TABLE 4

ANIMAL AND EXPERIMENT	HOURS AFTER ANESTHESIA		RECTAL TEMPERATURE	BLOOD PRESSURE	HEART RATE PER MINUTE	RESPIRATORY RATE	RESPIRATORY VOLUME IN CC. PER MINUTE	RESPIRATORY EXCHANGE		R. Q.	TOTAL CALORIES PER HOUR	PER CENT VARIATION FROM BASAL	PROGRESS NOTES	
								Cc. O_2 per minute	Cc. CO_2 per minute				Time	
a. Control experiments														
			°C.	mm. Hg.										
No. 27	1h	07m	39.49	100	150	30	568.1	25.56	16.82	0.658	7.17		0:25	Decerebration with slight hemorrhage
7/14/21	1	26	39.44	104	148	24	535.3	25.43	17.29	0.680	7.13			
	1	48	39.62	130	152	30	592.2	26.23	19.07	0.727	7.37			
Female	2	57	39.37	136	136	48	1211.0	28.23	22.90	0.811	8.10	+12.2	2:40–3:07	50 cc. 10 per cent urea injected
2.9 K	3	15	39.37	134	164	48	1566.0	26.31	23.33	0.887	7.70	+6.7		
	3	41	39.47	160	144	36	1596.0	28.09	22.50	0.801	8.04	+11.4	3:35–3:40	Slight movements
	4	00	39.38	105	140	30	1402.0	26.08	17.81	0.683	7.31	+1.2		
No. 40	1	15	38.33	140	168	54	706.4	20.84	16.32	0.783	5.94		0:31	Decerebration .
9/16/21	1	31	38.30	140	180	27	576.7	21.74	16.55	0.761	6.16			Slight hemorrhage
	1	46	38.42	140	168	30	595.9	23.42	17.04	0.728	6.58			
Female	2	23	38.22	145	168	24	581.7	22.39	17.16	0.767	6.35	+1.9	1:58–2:24	50 cc. Ringer's solution injected
2.7 K	2	40	38.27	148	174	24	613.0	22.99	17.10	0.744	6.48	+4.0		
	2	59	38.37	140	190	30	721.3	23.23	18.68	0.804	6.65	+6.7		
	3	15	38.33	120	110	30	748.3	21.85	17.28	0.791	6.24	+0.2		
b. Experiments with glycocoll														
No. 15	2	22	39.49	165		14	891.1	42.59	29.85	0.701	11.94		0:48	Decerebration with 10 minutes artificial
6/24/21	2	39	39.58	144		10	814.2	40.06	28.01	0.699	11.23			respiration
	2	58	39.60	106		12	1023.0	42.85	32.93	0.769	12.17			

Male	3	35	39.55	165		12	1095.0	51.15	35.82	0.700	14.34	+21.7	3:26–3:55	Glycocoll injected
3.8 K.	4	02	39.25	103		40	1285.0	52.82	43.18	0.818	15.18	+28.9	3:40	Slight movements
	4	30	39.33	84		*	1695.0	48.15	43.91	0.912	14.17	+20.3	4:30	Cheyne-Stokes respiration
													4:52	Respiration ceased
No. 16	1	23	38.35	126	236	72	885.3	23.73	16.11	0.679	6.65		0:30	Decerebration
6/27/21	1	43	38.35	110	238	68	1317.0	24.49	19.49	0.796	7.00			
	2	04	38.20	106	230	76	1872.0	23.41	21.91	0.936	6.93		2:15	Moved
Female	2	53	38.25	116	215	96	2700.0	28.62	25.11	0.877	8.35	+21.7	2:43–2:55	Glycocoll injected
3.0 K.	3	05	38.40	108	210	84	3239.0	28.50	24.29	0.852	8.27	+20.6	3:02	Moved
	3	17	38.60	120	210	75	4157.0	34.50	30.76	0.892	10.11	+47.4		
	3	29	38.52	104	?	40	2343.0	28.58	18.74	0.656	8.01	+16.8		Autopsy showed early pregnancy
No. 23	1	07	38.68	140	136	30	813.7	27.67	19.61	0.776	7.76		0:21	Decerebration
7/ 9/21	1	26	38.72	106	131	30	739.2	27.28	18.55	0.680	7.65		1:30	Moved. 1.59. Moved
	1	58	38.62	100	145	30	705.0	24.89	18.19	0.731	6.99		2:26–3:05	Glycocoll injected
Female	3	12	38.85	158	150	66	1928.0	32.96	32.77	0.994	9.85	+31.9	4:25	Abdomen slightly rigid
3.1 K.	3	32	38.95	140	150	72	4541.0	49.95	37.24	0.745	14.09	+88.6	4:56	Rigidity increases
	3	45	38.90	140	176	84	5400.0	54.00	43.74	0.810	15.49	+107.3		Dyspnea
	3	58	38.89	138	162	80	4277.0	39.78	38.07	0.957	9.96	+33.4		
	4	23	38.92	106	208	48	1930.0	28.82	26.44	0.917	8.50	+13.8		
	4	56	39.31	94	?	*	1001.0	31.73	21.22	0.669	8.90	+19.1	5:05	Convulsions. Death at 5:23
No. 42	1	06	38.31	110	150	30	1206.0	22.07	16.76	0.760	5.97		0:27	Decerebration
9/22/21	1	24	38.07	114	145	30	1225.0	21.32	18.38	0.862	6.20		0:38	Artificial respiration started
	1	39	38.10	115	145	30	1159.0	21.55	15.87	0.737	6.07			
Female	2	36	38.47	140	150	30	1153.0	24.44	19.94	0.816	7.02	+15.5	2:01–2:46	Glycocoll injected
2.0 K.	2	47	38.30	150	132	30	1290.0	26.31	22.57	0.858	7.64	+25.7	2:53–3:40	Natural, irregular respiration
	3	48	38.22	82	132	30	1714.0	24.51	21.43	0.874	7.15	+17.6		

TABLE 4—*Concluded*

ANIMAL AND EXPERIMENT	HOURS AFTER ANESTHESIA		RECTAL TEMPERATURE	BLOOD PRESSURE	HEART RATE PER MINUTE	RESPIRATORY RATE	RESPIRATORY VOLUME IN CC. PER MINUTE	RESPIRATORY EXCHANGE		R. Q.	TOTAL CALORIES PER HOUR	PER CENT VARIATION FROM BASAL	PROGRESS NOTES	
								Cc. O_2 per minute	Cc. CO_2 per minute				Time	
c. Experiments with glutamic acid														
			°C.	*mm. Hg*										
No. 30	1	34	38.94	114	156	24	416.9	22.89	12.76	0.557	6.42		0:33	Cerebral pressure applied
7/19/21	1	51	38.86	122	178	20	351.4	20.66	12.90	0.624	5.79			
	2	10	38.92	122	216	14	341.4	21.10	14.17	0.672	5.91		3:03–3:47	Glutamic acid injection
Female	3	38	39.04	190	230	24	1198.0	43.12	32.46	0.753	12.19	+101.9	3:02–3:33	Vomited
3.1 K.	4	02	39.80	175	230	66	1742.0	41.81	31.53	0.754	11.82	+95.8		
	4	26	40.44	130	280	46	823.4	31.45	21.90	0.696	8.82	+29.5	4:25	Cat cooled by fan
	5	18	40.45	88	196	48	513.1	23.75	16.88	0.711	6.66	+10.3		
	6	18	40.03	70	228	34	452.3	23.16	16.47	0.711	6.49	+7.5	6:15	Respiration irregular
No. 32	1	39	36.56	128	174	30	557.7	25.26	20.41	0.808	7.26		0:33	Cerebral pressure applied
7/22/21	1	55	36.46	128	180	24	529.9	26.76	20.40	0.762	7.58			
	2	12	36.54	140	184	24	544.7	26.58	19.50	0.734	7.48			
Male	3	03	36.12	140	180	28	491.2	27.56	18.32	0.665	7.73	+3.2	2:28–3:02	Glutamic acid injected
4.0 K.	3	21	36.50	150	200	24	486.3	29.42	19.16	0.651	8.25	+10.9		
	3	48	36.51	152	212	24	505.1	28.09	19.45	0.692	7.87	+5.8	2:58–3:02	Vomited
	4	25	36.60	168	224	30	541.1	28.08	19.91	0.709	7.87	+5.8	4:56	Spasmodic respiration

No. 41	1	55	37.85	115	208	48	889.8	27.67	18.86	0.682	7.76	0:28	Cerebral pressure applied
9/19/21/	2	14	37.82	118	216	44	887.6	25.21	18.55	0.736	7.09		
	2	32	37.92	115	240	36	831.1	23.27	18.03	0.775	6.62		
Male	3	17	37.23	130	180	42	675.8	23.86	16.69	0.700	6.69	−6.6	2:50–3:26 Glutamic acid injected
2.6 K.	4	25	37.97	100	240	32	878.6	25.57	16.61	0.649	7.17	+0.1	3:00 Vomited
	4	47	37.87	86	192	32	868.5	28.14	15.72	0.559	7.89	+10.2	3:45–4:05 Artificial respiration. Blood pressure 50 mm. Hg
No. 43	1	24	37.81	110	144	42	1764.0	24.35	19.41	0.797	6.96		0:30 Decerebration
9/24/21	1	38	37.84	100	144	36	1432.0	22.92	18.62	0.812	6.58		1:10–1:20 Very restless. Further decerebration
	1	53	37.80	110	126	32	1356.0	22.77	18.16	0.798	6.51		
Female	3	03	37.76	100	164	54	1888.0	23.98	20.39	0.850	6.95	+4.0	2:43–3:08 Glutamic acid injected. Walking reflex occasionally
2.4 K.	3	17	37.82	100	126	42	1784.0	23.02	19.63	0.853	6.68	+0.0	
	3	33	37.83	120	182	48	2198.0	23.52	19.56	0.832	6.79	+1.6	

TABLE 5

ANIMAL AND EXPERIMENT	HOURS AFTER ANESTHESIA	RECTAL TEMPERATURE	BLOOD PRESSURE	RESPIRATORY RATE	RESPIRATORY VOLUME IN CC. PER MINUTE	RESPIRATORY EXCHANGE		R.Q.	TOTAL CALORIES PER HOUR	PER CENT VARIATION FROM BASAL	PROGRESS NOTES	
						Cc. O_2 per minute	Cc. CO_2 per minute				Time	
a. Urethane												
		°C.	*mm.Hg.*									
No. 3	1h 34m	38.40	118	41	367.5	19.70	13.93	0.707	5.52			
4/4/21	1 58	38.58	124	46	505.4	21.99	16.98	0.772	6.25			
	2 26	38.60	106	56	472.0	20.96	16.76	0.800	6.00			
Female	3 46	38.75	156	40	573.5	25.35	20.19	0.796	7.25	+22.4	3:23–3:42	Glycocoll injected
2.8 K.	4 11	38.87	158	38	396.6	21.69	18.88	0.870	6.32	+6.8		
	4 32	38.84	128	41	531.3	22.74	20.40	0.877	6.64	+12.2		
	4 47	38.60	116	44	455.3	20.35	17.94	0.881	5.95	+0.5		
No. 4	1 20	37.10	102	35	435.7	21.61	15.16	0.702	6.06		0:35	5 cc. more urethane given
4/18/21	1 44	37.03	110	42	513.8	21.73	15.52	0.714	6.09			
	2 08	37.02	112	36	559.0	21.63	16.60	0.767	6.14		2:08–2:15	Tremor
Male	2 59	36.98	130	29	598.9	22.04	21.14	0.959	6.56	+7.5	2:56–3:03	Glycocoll injected
3.5 K.	3 17	37.13	128	29	583.8	23.06	19.61	0.851	6.69	+9.7		
	3 45	37.23	122	22	477.0	23.80	17.84	0.750	6.72	+10.2	3.35	Vomited
	4 28	36.67	140	24	639.7	23.09	20.92	0.906	6.79	+11.3		
No. 8	1 46	36.91	106	150	1544.0	20.68	13.58	0.657	5.80			
6/6/21	2 07	36.80	92	154	1695.0	21.35	14.23	0.667	5.99			
	2 21	36.93	88	168	1794.0	19.74	14.53	0.736	5.55			

Female	3	07	36.76	128	68	675.0	18.43	16.40	0.890	5.40	−6.6	2:49–2:56	Glycocoll injected
2.5 K.	3	28	36.90	116	66	841.5	20.87	18.01	0.863	6.07	+5.0	3:37–3:55	Glycocoll injection repeated
	4	04	37.02	126	55	681.5	20.44	16.63	0.813	5.87	+0.2		
	4	18	37.10	104	38	534.4	18.28	14.11	0.772	5.19	−11.2		
No. 9	1	52	37.77	148	70	722.1	18.85	14.37	0.762	5.34		1:30	Overheated
11/1/20	2	20	37.55	126	36	467.3	19.02	13.97	0.735	5.35			
	2	40	37.45	108	?	466.6	16.98	13.30	0.783	4.84			
Female	3	30	36.66	142	?	382.0	17.61	12.95	0.735	4.95	−4.4	3:05–3:23	Glycocoll injected
2.4 K.	3	50	36.84	124	65	453.3	17.63	14.32	0.812	5.06	−2.3		
	4	20	36.93	134	49	370.5	17.19	11.45	0.666	4.82	−6.9		

b. Paraldehyde

No. 11	1	58	38.47	92	54	1131.0	25.86	18.99	0.740	7.23			
6/9/21	2	16	38.48	96	44	1099.0	24.51	20.22	0.825	7.06			
	2	38	38.48	82	48	1191.0	23.58	20.13	0.854	6.84			
Male	3	28	38.61	148	48	1041.0	27.18	23.85	0.877	7.93	+12.6	3:02–3:18	Glycocoll injected
2.4 K.	3	51	38.63	114	52	1065.0	26.42	24.18	0.915	7.78	+10.5		
	4	10	38.54	96	54	1320.0	25.88	24.82	0.937	7.67	+8.9		
No. 12	2	41	35.93	106	96	965.8	15.93	11.01	0.691	4.47			
6/20/21	3	09	36.11	100	60	568.5	12.62	8.93	0.707	3.54			
	3	29	36.08	88	72	535.5	13.76	10.66	0.774	3.91			
Female	4	12	35.90	88	64	556.2	15.91	13.18	0.829	4.59	+15.6	3:50–3:57	Glycocoll injected
2.1 K.	4	33	35.96	86	66	470.5	13.26	11.10	0.837	3.83	−3.5	4:55	Respiration ceased

probably higher than those observed because in some cases the probable height of metabolic rise was missed, and in others the metabolism was still higher than the basal value when the experiment had to be discontinued.

b. Glutamic acid. In order to compare the results of this method with those secured per os by Lusk and others, four more experiments were performed in which glutamic acid was substituted for glycocoll. In three out of four experiments no appreciable specific dynamic action occurred. The first experiment did, however, show an unusually large increase in metabolism (49 per cent); but this experiment may be discounted because the cat had a high and rising temperature, a very low respiratory quotient at the outset, high blood pressure and heart rate, and a rigid thorax after injection.

CONCLUSIONS

These experiments indicate the following conclusions:

1. The intravenous injection of 5 grams of glycocoll produces a definite specific dynamic action in the decerebrate cat.

2. Glutamic acid in equal amounts produces no increase in the rate of total metabolism in three out of four experiments.

3. No increase in metabolism appears when glycocoll is injected intravenously into cats anesthetized by urethane or paraldehyde.

4. The decerebrate preparation offers a means for further investigation of the mechanisms involved in the specific dynamic action of protein.

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A QUANTITATIVE STUDY OF A SALIVARY CONDITIONED REFLEX

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Collins and Tatum (1925) discovered an interesting conditioned reflex during their study of the effects of daily injections of morphine on the dog. They found that after seven or eight injections their animals would salivate not only after the injection, but also before morphine was administered to them. The entrance of the experimenter into the room where the animals were kept was sufficient to call forth profuse salivation, and at times emesis. This salivary conditioned reflex persisted as long as the daily injections were kept up. We had a chance to see these dogs salivating, and it occurred to us that this reflex might be used to test Pavlov's theory of sleep.

In summarizing the results obtained in his laboratory during a score of years of investigation of the nature of conditioned reflexes, Pavlov (1923) describes, among many other types, a salivary conditioned reflex that he terms a delayed reflex. This type of reflex is developed by applying the conditioned stimulus for some time before applying the unconditioned one. When the animal (dog) learns to respond to the conditioned stimulus alone, it does so at first immediately upon the application of this stimulus, but on further training it gradually begins to respond with a greater and greater delay until finally it comes to respond with a delay almost exactly equal to the interval of time that separates the application of the conditioned stimulus from that of the unconditioned. The most remarkable thing observed in connection with this delayed conditioned reflex was a tendency on the part of the dog to fall asleep during the "delay." According to Pavlov the delay of the response under these special conditions is brought about by an inhibition of the salivation that would otherwise be set off at once after the conditioned stimulus is applied, and he thinks that the spreading of this inhibition from a small area (the center for salivation) to the entire cortex is responsible for the onset of sleep. We thought that the Collins-Tatum reflex offered an opportunity to associate the injection of morphine with a certain definite set of conditions, which could be maintained for an hour or two before the actual injection, and to see if the animal would fall asleep or become somnolent during the interval.

A secondary purpose we had in mind was to determine the manner in which this reflex developed and became extinguished. It will be seen that we failed completely in our main object, but that we obtained interesting results in our quantitative study of the reflex.

METHOD. As we could not be sure that none of the saliva secreted was swallowed by the dogs, it was necessary to prepare animals with salivary fistulae, and to collect the saliva as it came out of the duct. We had no idea of how such fistulae are prepared, and so we developed a technique of our own, which is very simple and may well be the same employed by others. We used the duct of the submaxillary gland, since it could be brought out directly under the lower jaw, making the collection of saliva easy. At the front end of the floor of the mouth there are two papillae lying close together, one on each side of the midline. These papillae contain the terminals of the ducts of the submaxillary glands. According to Chauveau (1903), the sublingual gland is not present in the dog, but there is a small accessory gland, along Wharton's duct, with its own excretory duct which opens into the same papilla as Wharton's. In the operation the mucous membrane of the floor of the mouth is cut just in front of one papilla, and a flap of mucous membrane 4 to 5 mm. wide and 2 to 3 cm. long, containing the duct, is dissected out. This is not difficult, because the duct lies very superficially and can be traced along the floor of the mouth for some distance. Some bleeding, often arterial, occurs during this resection, but it generally stops after a while. A thread is now tied around the end of the flap *in front* of the papilla, and the other end of the thread is attached to the grooved head of a sharp probe. The probe is thrust through the floor of the mouth at the root of the flap of mucous membrane, and when the probe is pulled out under the jaw it brings out the end of the flap by means of the string attached to it. The flap should project about 5 mm. and should remain outside without undue traction. The end of the flap is bent backward, without twisting, and stitched to the skin. The anterior part of the duct is thus curved downward and backward, and the papilla is on the under surface of the jaw. No stitches are needed in the mouth. The wound heals in a few days, and there is no tendency for the papilla to be retracted. If, however, the flap cut out is not long enough and has to be stretched before it can be fixed under the jaw, it will retract as the wound heals, and the fistula will close. No asepsis is required for this operation.

To make it certain that the presence of the observer would have no effect upon the dogs we kept them in cages placed in our room. We let them run about the room at frequent intervals and played with them, without at any time calling forth a secretion of saliva, even when the conditioned reflex was fully established. As a conditioned stimulus we employed the following set of conditions. The dog was taken out of his

cage and placed in a stock, such as is used for collecting gastric or pancreatic juice from a dog with an abdominal fistula. A thistle tube was tied around the lower jaw, just behind the canine teeth, leaving the upper jaw free, so that the animal would have no difficulty in vomiting, if need be. The papilla projecting from the jaw was right over the center of the thistle tube, the stem of which was cut very short and was slipped through a hole in a small size rubber stopper, inserted into a 15-cc. graduated centrifuge tube. All the saliva coming from the fistula got into the graduated tube through the thistle tube. This set of conditions always preceded an injection of morphine. The animals were left in the stock for a period of time, constant for the given dog, but varying in the case of different dogs, from 15 minutes to 2 hours, at the end of which they received a subcutaneous injection of morphine. Very rarely, and for a special reason each time, the dogs were kept in the stock for longer periods of time before morphine was injected. After the administration of the drug the animals were left in the stock as long as they continued to secrete saliva. One of us remained with the dog throughout the test and made readings of the centrifuge tube every 5 minutes, both before and after the injection of morphine. The graduated tube held only 15 cc. and had to be changed frequently, but the dogs were accustomed to our manipulations and were in no way affected by them. The injections were repeated daily, including Sundays and holidays, and in some cases every two days. The tests were made in the morning or in the afternoon or in the evening, always at about the same time for a given dog. The animals were generally fed after the test. Each of us acted as observer, but there was no regular alternation, and the dogs were used to the presence of one or both of us at all times. The morphine used for injections was a 1 per cent solution of morphine sulfate, to which we shall hereafter refer as morphine.

Results. We are presenting here the data obtained on eight dogs which we had in the laboratory for nearly a year. Each of them was used for one or more series of tests, the behavior of the dogs in subsequent series of tests having been modified by the preceding series in some respects, but left unaffected in others. To make the delay between the placing of the dog in the stock and the injection of morphine sufficiently long to let the dog fall asleep, we made this period equal to two hours in the case of our first dog. We did not measure the saliva secreted by this dog in the two hours preceding the injection for the first six days. When we began to collect saliva from the fistula we found that the dog had already developed a conditioned secretion of 22.1 cc. in two hours. The complete data on this dog, "Whitie," are given in table 1. It will be seen that the amount of saliva secreted in two hours increased rapidly from day to day, until ten days after the beginning of the experiment it reached a maximum of 99.9 cc. With minor fluctuations the dog kept up

TABLE 1

The development and extinction of the salivary conditioned reflex in "Whitie"

DATE	I	II	III	REMARKS
October				
30	22.1	??	??	Injections of morphine begun on October 24 (daily 40-50 mgm.)
31	44.8	0.7	0.7	
November				
1	53.4	5.1	5.0	
2	80.5	9.5	9.4	
3	99.9	6.6	6.5	
4	86.6	1.6	1.6	Dog very sleepy. Fought injection
5	87.4	5.4	5.4	Vomited twice before injection. Very sleepy and fully relaxed
6	97.9	3.3	3.3	Vomited twice before injection. Refuses food after injection, walks with a drunken gait
7	100.3	6.8	6.8	
8	100.7	??	??	
9	90.5	3.9	3.9	More restless than usual
10	95.5	5.4	5.4	
11	102.8	??	??	
12	93.8	6.6	6.6	
13	91.7	15.1	15.1	Defecated after injection
14	92.3			Daily injections of morphine discontinued
15	29.6			
16	2.9			
17	3.2			
18	1.8			Sleepy
19	1.5			
20	0.0			5 cc. of 0.9 per cent of NaCl solution were injected into back of animal at 9:15. During the next 15 minutes 1.6 cc. was secreted
21	0.1			5 cc. of 0.9 per cent NaCl injected. In 15 minutes 2.3 cc. of saliva were secreted. When put in cage dog eats with relish
January				
6	0.0	0.6	0.6	Morphine injections resumed (40 mgm. daily) Defecated after injection. Weights 11.7 kilos
7	1.0	5.4	4.2	Vomited after injection
8	1.1	5.5	5.3	
9	3.5	2.8	2.8	
10	7.0	5.8	5.8	Retches after injection
11	7.1	4.1	4.1	
12	16.4	5.7	5.7	Vomited after injection
13	4.4	7.1	6.6	
14	17.9	8.3	8.3	Vomited after injection
15	13.8	6.2	6.2	
16	11.5	10.1	10.0	Vomited after injection

TABLE 1—*Concluded*

DATE	I	II	III	REMARKS
January				
17	13.7	7.5	7.5	Vomiting and prolonged retching after injection
18	9.1	7.6	7.5	Vomited after injection
19	12.8	3.6	3.6	
20	17.1	5.9	5.9	
21	21.8	9.3	8.5	
22	23.3	8.5	8.5	Vomited after injection
23	19.7	8.1	8.0	Fed at 8:00. Vomited before injection
24	6.6	1.7	1.7	Dog blindfolded during test. Dog remains motionless, except for wagging of tail
25	12.1	9.3	9.2	
26	12.8	9.0	8.7	
27	18.1	6.9	6.9	
28	5.1			Daily injections of morphine discontinued
29	1.5			
30	1.0			
31	0.0			
February				
1	0.2			
2	0.0			
3	0.0			
4	0.0			

First series of tests made in the evening, second series in the morning. Under I are given the quantities of saliva in cubic centimeters secreted in 2 hours in the first series, and in 1 hour in the second series, before morphine was injected. Under II are tabulated the quantities of saliva in cubic centimeters secreted after the morphine injection, and under III those portions of the latter which were secreted in 15 minutes after the injection.

the conditioned salivation at this level as long as the daily injections of morphine (40 to 50 mgm.) were continued, and that was ten more days. During the two hours spent in the stock in expectation of the injection the dog showed nothing peculiar in his behavior. At times he was lively, at others depressed or sleepy. Exactly the same behavior can be observed in any dog kept in a stock for any length of time. On two occasions, however, the dog vomited before he received the injection of morphine, something that was already observed by Collins and Tatum. After the injection of morphine the dog generally showed signs of excitement, followed at times by retching and vomiting. After 15 or 20 minutes the excitement passed off and was replaced by profound depression. At this time the dog usually stopped secreting saliva and was returned to his cage. When the injections of morphine were stopped, the dog was secreting over 90 cc. of saliva in two hours, but on the following day he

TABLE 2

The development and extinction of the salivary conditioned reflex in "Brindle"

Fistula prepared on December 11. Weight 8.5 kilos

DATE	I	II	III	REMARKS
December				
14	0.0	1.2	1.2	Morphine injections begun (50 mgm. daily). Struggles a little when first stocked. Panted violently after injection. Drunken gait. Refused food
15	0.1	1.3	1.3	
16	0.2	2.5	2.5	
17	0.4	0.7	0.7	
18	5.3	4.1	4.1	Panted, whined and behaved like a dog that has received morphine, before injection
19	11.2	5.2	5.2	Showed signs of distress, whined and panted before injection. Refused raw meat after injection
20	7.5	6.5	6.4	Whines and is restless before injection
21	23.7	5.3	5.2	Signs of distress immediately on being stocked. Pants before and after injection
22	10.5	5.5	5.5	
23	38.1	12.9	12.1	Whined before injection. Dog vomited for the first time after injection
24	44.9			Daily injections of morphine discontinued Distressed, panted, etc., as if he had received an injection. Animal eats raw meat heartily
25	17.5			Appeared sleepy and closed eyes. Eats voraciously after taken off
26	20.4			Whined
January				
13	0.2	0.6	0.4	Injections of morphine resumed (30 mgm. daily)
14	12.5	10.4	9.8	Panted, seemed distressed
15	26.2	11.0	9.5	Showed signs of distress, whined, panted before injection. Violent panting and whining after injection. Vomited after injection
16	44.9	4.4	4.4	Panted and whined almost continuously before injection. Vomited after injection
17	33.5	11.4	9.8	Panted and vomited profusely after injection
18	47.9	6.2	6.2	Whined and panted before injection
19	44.7	9.3	9.2	Began whining soon after being stocked
20	40.3	13.8	13.6	Panted before injection
21	41.0	9.6	7.1	Whined and panted before injection. Panted violently after injection
22	44.7	4.5	4.5	Whined before injection. Run for another hour. Secreted 40.5 cc. during the second hour
23	29.3	7.2	7.2	

TABLE 2—*Concluded*

DATE	I	II	III	REMARKS
January				
24	33.3	9.8	8.8	Whined and panted before injection. Panted and retched after injection
25	*16.9*	0.5	0.5	Had previously been blindfolded for 20 minutes, and was kept blindfolded during test in stock. Vomited after injection
26	42.9	5.2	5.1	Panted and whined before injection
27	42.3			Panted and whined. Daily injections of morphine discontinued
28	27.8			Panted and whined
29	37.6			Whined plaintively and panted from start
30	29.1			
31	24.7			Panted
February				
1	10.9			
2	15.0			
3	12.2			
4	5.2			Panted
5	8.0			Panted
6	0.3			
7	1.0			Panted for a short time
8	0.6			
9	1.1			
10	0.9			
11	1.2			
12	0.3			All secretion in last 10 minutes
13	1.8			
14	3.9			
15	0.1			

First series of tests made in the afternoon, second series in the morning. Under I are given the quantities of saliva in cubic centimeters secreted during 60 minutes before morphine was injected. Under II are tabulated the quantities of saliva in cubic centimeters secreted after the morphine injection, and under III those portions of the latter which were secreted in the first 15 minutes after the injection.

secreted only 29.6 cc., and on the third day 2.9 cc. That was a very precipitous return to normal, the reflex becoming practically extinguished in two days.

In the second dog, "Brindle," we took care to measure the quantity of of pre-morphine saliva from the very beginning. This dog was kept in stock for one hour before giving him morphine (50 mgm. daily), and already on the second day we obtained 0.1 cc. of saliva from the fistula. On the third day be secreted 0.2 cc., on the fourth 0.4 cc. (table 2). After this the conditioned reflex suddenly flared up, and after ten daily injections the volume of saliva excreted as a conditioned reflex amounted to 44.9 cc.

TABLE 3

The development and extinction of the salivary conditioned reflex in "Rusty," and the effect of an infection with mange upon the course of the reflex

Operated February 13, 1926

DATE	I	II	III	REMARKS
February				
17	1.1			
18	2.0			
19	0.3			
20	2.6	3.4	3.3	Morphine injections begun (30 mgm. daily). No undue struggling even during this first injection. Somewhat depressed. No vomiting or defecating
21	0.2	3.7	3.7	Moans and vomits after injection
22	2.1	4.8	4.7	Restless and excited before injection. Pants hard and vomits after injection
23	6.7	8.3	6.2	Panted violently. Whined and vomited mucus before injection
24	0.4	7.3	7.3	Vomited after injection
25	2.2	5.6	5.5	Vomited repeatedly after injection
26	4.8?	7.5?	7.2?	Started secreting immediately on being put in stock. Part of saliva lost. Vomited repeatedly after injection
27	1.4	5.9	5.4	Somnolent, eyes closed, before injection
28	2.0	5.3	5.3	Vomited repeatedly after injection
March				
1	2.2	5.5	5.5	Pants violently before injection
2	4.0	5.6	5.6	Vomited after injection
3	25.8	10.5	10.5	Vomited 5 times between 7 and 8 while other animals were being run. Saliva started running profusely from fistula, before thistle tube could be attached
4	19.2	7.4	6.6	
5	28.3	7.2	7.2	Sleepy, eyes closed before injection. Vomited. Panted after injection
6	28.8	6.5	5.8	
7	11.9	11.3	10.9	Eyes closed, sleepy, before injection
8	28.5	8.5	5.4	Vomited after injection
9	14.2	6.9	6.9	
10	36.2	7.5	7.5	
11	27.6	6.2	6.1	Sleepy, eyes closed before injection
12	28.2	12.7	10.0	Pants violently and continually, both before and after injection
13	34.6	7.4	7.4	
14	32.0	7.7	7.5	Panted, sleepy before injection
15	28.3	6.6	6.5	
16	21.8?	8.5	8.4	
17	28.3	8.1	7.9	
18	35.9	7.4	7.4	Panted before and after injection
19	32.9			Daily morphine injections discontinued
20	8.4			
21	0.5			Appears sleepy and dozes

TABLE 3—*Concluded*

DATE	I	II	III	REMARKS
March				
22	0.0			Sleeping, snoring, relaxes, wakes up
23	0.0			
24	0.6			Pants
25	0.0			Falls asleep, relaxes, snores
26	0.0			Falls asleep, relaxes, snores
27	0.1			
28	0.0			
29	0.0			
30	0.0			
31	0.0			
April				
1	0.2	4.0	4.0	Daily injections of morphine resumed
2	0.2	4.3	3.2	
3	0.0	4.3	4.3	Vomits repeatedly after injection. Pants violently
4	0.1	4.3	4.3	
5	4.0	5.9	5.7	Vomits after injection
6	3.9	2.1	2.1	
7	2.8	4.0	4.0	
8	0.0	0.1	0.1	Vomits after injection
9	10.2	10.7	10.7	
10	10.9	8.5	8.4	
11	11.6	4.0	4.0	
12	6.2	4.6	4.5	Fed by mistake before the test. Vomits before and after injection
13	8.0	8.4	7.6	
14	3.4	4.7	4.7	
15	7.3	6.0	5.4	
16	18.2	5.8	5.7	Pants before injection
17	0.0	2.1	2.1	Pants after injection. Eats immediately after being placed in cage
18	0.4	9.0	6.8	Pants violently after injection
19	8.3	8.4	8.0	Prolonged retching and vomiting after injection
20	4.6	8.3	8.3	
21	7.6	5.9	5.9	
22	2.3	4.4	4.4	
23	10.1	4.2	3.4	
24	4.2	6.0	5.9	Vomits after injection
25	4.7			Well developed mange diagnosed. Morphine injections discontinued
26	0.9			
27	0.0			
28	0.0			
29	0.2			Very mangy

First series of tests made in the morning, second series in the afternoon. Under I are given the quantities of saliva in cubic centimeters secreted during 60 minutes before morphine was injected. Under II are tabulated the quantities of saliva in cubic centimeters secreted after the morphine injection, and under III those portions of the latter which were secreted in the first 15 minutes after the injection.

TABLE 4

The development and extinction of the salivary conditioned reflex in "Tramp," the effect of starvation upon the fully developed reflex, and the effect of realimentation on the further course of the reflex

DATE	I	II	III	REMARKS
March 31	0.0			
April 1	0.0	5.8	5.8	Morphine injections begun (30 mgm. daily). Vomited, defecated after injection
2	0.0	3.9	3.9	
3	0.4	6.4	6.4	
4	4.8	16.0	15.9	Whines before injection; vomited after injection
5	15.0	9.6	9.5	Whines before injection
6	25.9	9.5	9.5	Whines before injection
7	29.5	9.9	9.9	Several drops of saliva lost before tube could be attached. Run for another half-hour. Second half hour 22.8 cc.
8	22.1	8.0	7.9	Shivering before injection. Much noise in room
9	33.0	9.6	9.6	Whines before injection
10	35.9	10.5	9.8	
11	42.3	14.2	11.0	Whines and struggles before injection
12	33.4	21.4		Fed before test by mistake. Whines and struggles during test. Violent retching and vomiting before and after morphine injection
13	33.6	20.6	14.9	Whines before, pants after injection
14	31.9	13.5	10.6	Fights being stocked. Restless before injection
15	40.1			Morphine injections discontinued. Fights being stocked
16	28.1			Retching. Room very warm
17	30.8			Whines throughout test
18	14.5			Fights against being taken from cage
19	18.1			
20	11.6 (?)			Broke tube during last period so that the saliva for that period was not measured
21	8.2			
22	13.2			
23	10.9			
24	15.7			
25	8.3			
26	12.3			
27	4.1			
28	4.7			
29	6.0			Room very warm. Panting from heat
30	2.1			

TABLE 4—*Continued*

DATE	I	II	III	REMARKS
May				
1	1.2			
2	2.6			
3	1.2	7.7	7.7	Daily injections of morphine resumed. Retching and vomiting after injection
4	7.0	10.8	10.8	Vomited after injection
5	21.4	11.3	11.3	Vomited profusely after injection
6	29.5	7.5	7.0	Salivation started before dog was removed from cage
7	27.7	9.6	9.3	Whines before injection
8	29.5	10.2	10.1	Whines and struggles before. Fed for last time
9	28.2	10.1	9.9	Starvation begun. Morphine injections continued
10	27.2	5.7	5.7	
11	25.6	10.1	8.6	
12	26.1	9.0	8.7	Began to salivate before tube could be attached. Whines. Weight 8.7 kgm.
13	21.5	5.5	5.5	
14	17.9	5.9	5 8	Weight 8.5 kgm.
15	16.0	6.1	6.0	
16	12.1	6.2	6.2	Repeated violent retching and vomiting of saliva and bile after injection
17	6.0	1.4	1.4	
18	6.9	1.9	1.9	
19	6.8	2.6	2.6	Starvation ended. Morphine injections continued. Weight 7.7 kgm. Given food but would not eat. Ate part of food during night
20	5.8	2.4	2.4	
21	5.8	2.7	2.7	Weighs 8.5 kgm.
22	13.4	7.5	7.3	Whines after injection
23	15.2	4.3	4.3	Whines before injection. Weighs 8.7 kgm.
24	24.4	5.7	5.7	
25	25.9	4.9	4.9	
26	30.8	11.5	11.4	Retches after injection. Weighs 9.0 kgm.
27	39.2	8.5	8.5	
28	34.4	15.0	14.9	Vomited after injection
29	21.1	13.1	13.0	Salivates profusely before taken from cage
30	31.0	7.7	7.6	
31	23.8	8.3	8.2	
June				
1	10.8	7.9	7.9	Vomited after injection
2	23.1	9.6	9.5	Vomited and retched repeatedly after injection
3	11.1	6.9	6.9	Getting mangy
4	12.5	8.2	8.2	
5	16.3	6.0	6.0	Vomited after removed from stock

TABLE 4—*Concluded*

DATE	I	II	III	REMARKS
June				
6	14.7	5.7	5.7	
7	5.5	7.9	7.9	Repeated vomiting and retching after injection
8	10.8	7.1	7.1	Whines before and after injection. Vomited after injection. Saliva very viscid
9	39.2	19.0	19.0	Pants and whines after injection. Saliva is *much less* viscid than on June 8
10	20.4	10.1	10.1	Whines before injection; vomited repeatedly after injection
11	27.0	13.5	13.4	
12	23.4	8.5	8.5	
13	13.0	11.4	8.8	
14	8.7	10.8	10.3	
15	16.4			Morphine injections discontinued
16	7.2			
17	2.3			
18	6.7			
19	0.3			
20	0.4			
21	0.0			

Tests made in the afternoon. Under I are given the quantities of saliva in cubic centimeters secreted in 30 minutes before morphine was injected. Under II are tabulated the quantities of saliva in cubic centimeters secreted after the morphine injection, and under III those portions of the latter which were secreted in 15 minutes after the injection.

in one hour. At this time the dog manifested most acute distress immediately upon being placed in the stock. He whined and panted, his eyes were protruding, and in general he behaved just the way a dog should behave a few minutes *after* receiving an injection of morphine.

Our third animal, "Rusty," was very slow in developing the reflex. This dog happened to secrete a little saliva (1 to 2 cc. in an hour) spontaneously, before we began to give him morphine. He is the only dog in whom we observed a semblance of "continuous" secretion from the fistulas, "sensible" enough to be collected and measured. After ten daily injections the reflex showed a sudden improvement, and with some fluctuations the secretion mounted to 30 cc. in one hour. "Rusty" is a very phlegmatic dog, and he appeared somnolent all the time, both when the conditioned reflex was at its height and when it was completely extinguished (table 3).

"Tramp," kept in the stock for only 30 minutes before the injection of morphine, began to secrete after two administrations of morphine (30 mgm. daily). The secretion rate gradually increased and was equal to 33 cc. after 8 injections. Then it continued to rise more slowly (table 4).

TABLE 5

The development and extinction of the salivary conditioned reflex in "Mother," the development of the reflex by alternate injections of morphine, the development and extinction of the reflex during starvation, the development and course of the reflex during starvation, and the effect of realimentation on the further course of the reflex

DATE	I	II	III	REMARKS
April				
3	0.0	34.1	0.1	Morphine injections begun (30 mgm. every 2 days)
5	0.4	5.7	5.3	
7	0.4	5.0	5.0	
9	2.9	5.2	5.1	
11	0.6	4.1	4.0	
13	8.1	12.5	10.2	Some saliva lost. Whined after injection
15	21.9	21.9	10.2	
17	21.8	18.3	14.7	Whined before injection
19	22.0	17.4	14.5	Panted and whined after injection
21	20.6	12.3	12.3	
23	31.8	10.1	10.0	
25	36.5	20.3	10.4	
27	35.7	12.6	12.4	
29	44.6	12.9	9.6	
May				
1	45.3			Injections of morphine discontinued
3	11.0			
5	3.1			
7	0.0			
9	0.0			
11	0.0			
12	0.0	2.1	2.1	Alternate injections of morphine—30 mgm. every other day. Dog placed in stock daily Panted after injection
13	0.1			No morphine
14	0.0	47.1	11.2	
15	0.0			No morphine
16	27.5	22.8	10.1	Panted and whined after injection
17	24.2			No morphine
18	13.2	18.7	18.4	Shivers before injection
19	17.1			No morphine
20	22.3	24.2	17.3	
21	29.9			No morphine
22	16.3	46.6	17.5	Whined and panted after injection
23	21.6			No morphine
24	27.5	57.4	19.8	
25	28.1			No morphine
26	30.0	44.4	18.2	Panted after injection
27	35.8			Injections of morphine discontinued
28	41.0			
29	18.2			
30	24.6			
31	20.1			

TABLE 5—*Continued*

DATE	I	II	III	REMARKS
June				
1	0.9			
2	0.6			
3	0.0			
4	0.0			
5	0.0			Fed for the last time
6	0.0			Starvation period begun
7	0.0	2.2	2.2	Morphine injections resumed (40 mgm. daily) Weight 13.5 kgm.
8	0.0	1.6	1.6	Whined after injection
9	0.0	5.4	5.3	Whined after injection
10	6.7	7.6	7.5	
11	10.9	14.7	14.6	
12	21.3	11.6	11.0	
13	22.3	26.5	15.0	Whined after injection. Weight 12.5 kgm.
14	24.3	10.8	10.3	
15	31.4	16.5	16.4	
16	31.4	15.9	9.2	Weight 11.9 kgm.
17	32.7	22.0	14.3	
18	30.3			Weight 11.5 kgm. Morphine injections discontinued. Starvation kept up
19	32.0			
20	1.3			
21	5.4			
22	0.0			
23	0.0			Weight 10.9 kgm.
24	0.0	0.9	0.9	Morphine injections resumed. Starvation continued
25	0.0	4.1	4.1	
26	0.0	0.5	0.5	Weight 10.4 kgm.
27	5.8	4.8	4.7	Whined after injection
28	6.7	8.2	5.8	Whined pitifully after injection Weight 10.2 kgm.
29	8.5	10.9	9.9	
30	6.3	5.0	5.0	Weight 10 kgm.
July				
1	5.9	7.8	7.7	Weight 9.9 kgm.
2	10.3	5.7	5.7	
3	12.0	5.0	4.9	
4	5.0	7.7	7.3	Weight 9.5 kgm.
5	0.0	1.8	1.8	
6	10.3	4.4	4.4	Weight 9.2 kgm.
7	4.5	3.1	3.1	
8	3.5	2.2	2.2	Weight 9.1 kgm. Realimentation begun. Offered some fresh hamburger after test, but she refused
9	21.4	5.9	5.9	Very hot day. Panted after injection
10	10.0	10.5	10.2	Weight 10.4 kgm.

TABLE 5—*Concluded*

DATE	I	II	III	REMARKS
July				
11	42.5	8.3	7.9	Weight 10.3 kgm. Defecated twice before injection. Weight 10.3 kgm.
12	31.6	7.3	7.2	Weight 10.2 kgm.
13	36.4	13.7	13.0	Weight 10.7 kgm.
14	37.9	17.9	14.0	
15	47.9	12.8	10.0	Weight 11.5 kgm.
16	51.0	15.4	13.9	Panted before. Given light anti-mange treatment
17	50.8			Panted heavily before injection
18	0.8	4.4	4.4	Panted after injection. Dog had not been given any water since experiment of yesterday
19	37.6	19.2	16.0	
20	55.1	18.4	15.4	Panted before injection
21	49.6	24.1	16.5	Panted before injection. Weight 12.2 kgm.
22	43.5	22.9	19.2	Panted after injection
23	45.6	14.2	12.6	
24	38.5	23.2	18.9	Weight 12.5 kgm.
25	42.7	16.7	13.7	Weight 12.6 kgm. Not fed nor watered for next two days
26	21.8	9.9	9.2	Weight 12.20 kgm.
27	10.3	8.6	7.7	Weight 11.7 kgm. Fed and watered after test
28	37.2	37.6	23.0	Weight 12.88 kgm.
29	53.1			Daily morphine injections stopped
30	29.2			
31	0.5			
August				
1	0.0			
2	0.0			

Tests made in the afternoon. Under I are given the quantities of saliva in cubic centimeters secreted in 30 minutes before morphine was injected. Under II are tabulated the quantities of saliva in cubic centimeters secreted after the morphine injection, and under III those portions of the latter which were secreted in 15 minutes after the injection.

Our prize dog was "Mother" who had raised a litter of pups in our laboratory, prior to her being used for this work, and who was on very friendly terms with us. She is the only animal we ran continuously for four months. In the first series of tests we placed her in the stock every two days, instead of daily as was the rule for all other dogs. We wanted to see if one stimulus in 48 hours was sufficient for the establishment of the conditioned reflex. She began to secrete conditionally after one injection. She showed little improvement at first, but after five injections of morphine the rate became greatly accelerated, and after 14 injections she secreted 45.3 cc. in 30 minutes (table 5).

In all these dogs the amount of saliva secreted daily in the pre-morphine period increased according to a very definite rule. The whole period of development of the conditioned reflex may be divided into three phases. In the first of these there was very little increase in secretion from day to day; in the second the daily increase was by leaps and bounds; in the third there was a slowing down in rate at which the secretion increased, the reflex reaching a maximum value for the dog and for the time spent in the stock. When the quantities of saliva (in cubic centimeters) secreted as a conditioned reflex are plotted against the time in days, the curve is at first concave upward, then it becomes convex upward, and as it approaches the maximum, gets to be nearly a horizontal line. In other words, it shows a positive acceleration for the first half of its course and a negative acceleration for the second half (fig. 1), and assumes the appearance of an S-curve.

If the conditioned reflex is developed again in an animal in whom it had but recently been completely extinguished, it can be noted that the secretion reaches its maximum in a much shorter period of time. In "Brindle" it took ten injections of morphine (Dec. 14–24) to bring the secretion up to 44.9 cc. in one hour in the first series of tests, but the secretion rose to a similar level after only three injections (Jan. 13–16) in a second series when the reflex was reëstablished (table 2). "Tramp" secreted 29.5 cc.

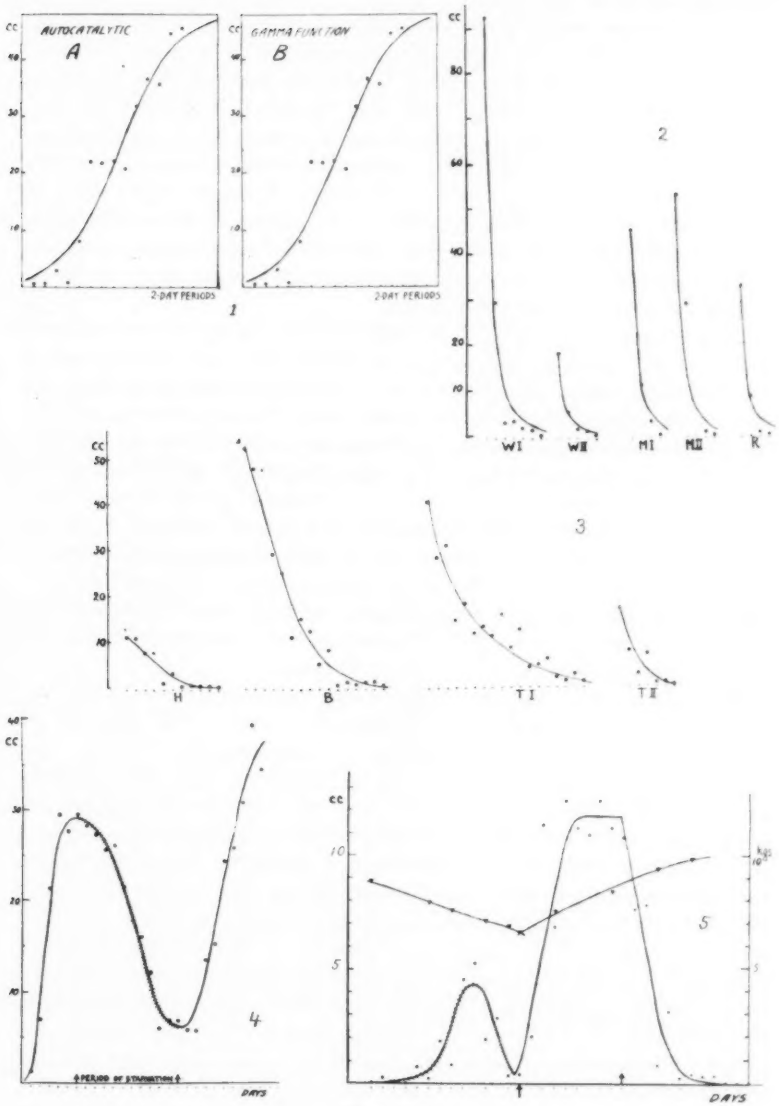
Fig. 1. The curve of development of the salivary conditioned reflex in "Mother." *A*, the curve for autocatalyzed monomolecular reactions, and *B*, the Gamma function curve, fit the observed values (given in table 12) equally well. Each curve is characterized by a concavity upward (positive acceleration) for the first half, and by a convexity upward (negative acceleration) for the second half of its course.

Fig. 2. The curves of extinction of the salivary conditioned reflex in "Whitie" (two curves, *WI* and *WII*). "Mother" (two curves, *MI* and *MII*) and "Rusty." All these curves are second degree parabolas. The dots in the abscissa represent successive tests after discontinuing the injections of morphine. For numerical data see table 13.

Fig. 3. The curves of extinction of the salivary conditioned reflex in "Hobo," "Brindle" and "Tramp," (two curves, *TI* and *TII*). All curves are characterized by a concavity upward (negative acceleration). The dots in the abscissa represent successive days after discontinuing the morphine injections. For the numerical data see tables 2, 4 and 6.

Fig. 4. The effect of starvation on the course of the conditioned reflex in "Tramp." Marked depression of reflex by starvation, followed by recovery on re-alimentation. Dots in abscissa represent successive days. Numerical data are given in table 4.

Fig. 5. The effect of starvation upon the development of the salivary conditioned reflex in "Hobo," and the effect of re-alimentation on the practically extinguished reflex. The dots in the abscissa represent successive days. The lower curve is for the secretion of saliva (*cc*), the upper curve is for the weight (*kg*) during and after starvation. At the first arrow re-alimentation was begun, while continuing the morphine. At the second arrow morphine injections were discontinued. For data see table 6.


AUTOCATALYTIC
A
GAMMA FUNCTION
B
2-DAY PERIODS
2-DAY PERIODS
1
2
WI
WII
MI
MII
R
3
H
B
TI
TII
4
PERIOD OF STARVATION
DAYS
5
DAYS

Figs. 1–5

in 30 minutes after six injections (Apr. 1–7) when run for the first time (table 4), but on repetition he secreted exactly the same amount in 30 minutes after only three injections of morphine. "Mother" (table 5) received ten injections of morphine before she secreted 31.8 cc. in 30 minutes when the conditioned reflex was developed in her for the first time (Apr. 3–23), whereas on reëstablishment of the reflex, under unfavorable conditions, she secreted 27.5 cc. after only two injections (May 16). It is seen that the initial portion of the S-curve is absent in the curve of development of the conditioned reflex in an animal in whom the reflex had once previously been established: the curve does not linger along the axis of abscissae, but goes straight up and toward the end shows the usual slowing down or negative acceleration.

A dog not previously used, "Lad," was placed in the stock for 15 minutes daily and was given an injection of morphine (30 mgm.) at the end of the 15-minute period every other day. It was interesting to find out whether this alternate application of the unconditioned stimulus and the daily application of the conditioned stimulus would lead to the development of the conditioned reflex. We found that it did, but the reflex was very slow in appearing. The secretion never reached a definite maximum, fluctuated considerably from day to day, and yet we collected from the fistula as much as 10.6 cc. in one test, which for a 15-minute period is not a negligible amount of saliva. We made a similar series of tests on "Mother" (May 12–26) who had been run previously, and the reflex developed very promptly. Here, too, the daily fluctuations were considerable, but the conditioned secretion was abundant at all times.

The rate at which the conditioned reflex was extinguished, when the daily injections of morphine were discontinued, was not the same for all the dogs. Some behave like "Whitie" in this respect. "Rusty," for instance, secreted 32.5 cc. in one hour on March 19, when the injections were stopped (table 3). On the following day he secreted only 8.4 cc., on the third day 0.5 cc., and after that nothing. "Mother" secreted 45.3 cc. on May 1, when the administration of morphine was discontinued, and next time she was placed in the stand, in her case two days later, she was down to 11.0 cc., then to 3.1 cc., then to nothing (table 5). The curves of abolition of the conditioned reflex for these three dogs are given in figure 2. It will be seen that they resemble each other in that they all show marked negative acceleration. The same dog, when run twice, shows the same type of curve of disappearance of the reflex, whereas the curves of establishment and reëstablishment of the reflex are not the same in respect to their initial course. In other dogs, "Brindle," "Tramp" and "Hobo," the reflex disappears slowly. Instead of three or four days, it took these dogs from one to over two weeks to stop secreting saliva after the morphine injections were stopped. The figures obtained on these

TABLE 6

The development and the course of the salivary conditioned reflex during starvation in "Hobo," and the effect of realimentation on the further course of the reflex. Extinction of reflex upon discontinuance of morphine injections

Under-nourished at the time of operation. Not fed after operation, until after testing effect of starvation on the secretion had been studied. Weight 9.0 kgm. at time of operation. Weight when next fed 6.6 kilos.

DATE	I	II	III	REMARKS
May				
13	0.0			Struggles before. Weight 9.0 kgm.
14	0.0			Broke tube
15	0.0			
16	0.0	1.8	1.8	Morphine injections begun (30 mgm. daily) Starvation continued. Retching after injection. Weight 8.8 kgm.
17	0.2	2.4	2.4	
18	0.0	1.8	1.8	Broke tube. Whines after injection
19	0.0	4.9	4.9	Vomited hair and feces after injection
20	0.7	4.2	4.2	
21	0.2	3.6	3.6	Weight 7.9 kgm.
22	1.8	2.2	2.2	
23	0.8	2.8	2.8	Weight 7.6 kgm.
24	4.5	4.4	4.2	
25	5.2	0.3	0.3	
26	1.9	5.0	5.0	Retches after injection. Some bile in saliva drippings, but no vomiting. Weight 7.2 kgm.
27	2.8	1.1	0.9	
28	0.3	2.2	2.2	Weight 6.8 kgm.
29	0.4	1.3	1.3	Starvation ended; morphine injections continued. Weight 6.6 kgm. *Fed.* Eats immediately. Salivated profusely while eating
30	2.0	3.9	3.9	
31	11.3	5.9	5.8	Vomited before injection
June				
1	6.8	4.8	4.8	
2	12.4	7.5	7.2	
3	11.2	9.9	8.4	Vomited after injection
4	10.9	9.2	9.2	
5	12.4	10.4	9.8	
6	11.2	10.2	10.2	
7	10.8			Morphine injections discontinued
8	7.6			
9	7.8			
10	0.8			Weight 9.4 kgm.
11	3.1			Warm sultry day
12	0.2			
13	0.4			Weight 9.8 kgm.

TABLE 6—*Concluded*

DATE	I	II	III	REMARKS
June				
14	0.3			
15	0.3			
16	0.0			
17	0.0			
18	0.0			
19	0.3			
20	0.0			
21	0.0			Weight 9.8 kgm.

Tests made in the afternoon. Under I are given the quantities of saliva in cubic centimeters secreted in 15 minutes before morphine was injected. Under II are tabulated the quantities of saliva in cubic centimeters secreted after the morphine injection, and under III those portions of the latter which were secreted in the first 15 minutes after the injection.

animals are plotted in figure 3, and the curves all show a concavity upward, resembling in this respect the curves for the abolition of the reflex in the other dogs: the process is merely protracted.

During the work some of our dogs developed a mange infection. It was difficult to establish conditioned salivation in these animals, and if the reflex was fully developed at the time of the infection, it had a tendency to become extinguished, in spite of the fact that the daily injections of morphine were kept up. At the same time the dogs became extremely emaciated. It was interesting to see if the inanition itself had any effect upon the reflex. Froloff (1925) reported that long-continued undernutrition acted deleteriously on the salivary conditioned reflex. We therefore developed conditioned salivation in "Tramp" for the second time, and when the reflex reached a considerable magnitude, we began to starve the dog, at the same time keeping up the morphine injections. He was starved from May 9 until May 19, and in that period the conditioned secretion fell from 28.2 cc. to 6.8 cc. per 30 minutes (table 4). Realimentation was begun, the injections of morphine being continued, and in eight days the secretion rose to 39.2 cc. in 30 minutes, a higher level than that which was reached when the starvation was resorted to. The results are represented graphically in figure 4. We next decided to see if the reflex could be developed in a starving animal. We began to starve two dogs with salivary fistulae, never used previously, and gave them daily injections of morphine at the same time. The first, "Hobo," run for 15 minutes, developed the reflex rather slowly, reaching the height of 5.2 cc. after eight daily injections of 30 mgm. of morphine each (table 6), but the secretion began to diminish till after 13 days of fasting it was only 0.4 cc. Without stopping the morphine injections realimentation was begun,

TABLE 7

The development and course of the salivary conditioned reflex during starvation in "Goldie," and effect of realimentation on the further course of the reflex. Extinction of reflex upon discontinuance of morphine injections

DATE	I	II	III	REMARKS
April				
27	0.1			Weighs 13 kgm. Starvation started
28	0.4			
29	1.4			Struggles a great deal. Room very warm. Weight 12 kgm.
30	1.0			
May				
1	0.4	4.4	4.3	Morphine injections begun (30 mgm. daily) Vomits after injection
2	0.6	5.3	5.2	
3	0.1	18.9	4.0	Vomits hair after injection. Pants
4	0.4	6.8	6.7	Weight 10.9 kgm.
5	0.0	16.9	6.1	
6	1.8	10.1	7.9	Eyes are blood-shot after injection
7	5.8	19.1	8.7	Vomits after injection. Pants violently
8	4.8	16.5	10.0	Vomits foamy mucous substance after injection
9	6.2	7.9	6.1	
10	5.1	11.4	6.7	Pants and shows signs of distress before injection. Vomits foamy saliva after injection; pants continually. Discontinued, did not stop secreting
11	3.9	7.4	7.0	
12	4.8	10.8	5.8	Pants after injection
13	5.6	6.3	6.2	Pants before and after injection
14	6.8	?		Pants immediately on being stocked. Fistula outside of tube, part lost after injection
15	5.8	5.6	4.9	
16	6.6	5.0	4.0	Pants before and after injection Weight 9.7 kgm.
17	7.3	7.3	6.6	
18	3.1	4.7	4.5	
19	1.1	3.2	3.2	Starvation ended; morphine injections continued. Weight 9.3 kgm. Given food, but did not eat. Probably ate some of it the following night
20	7.3	2.4	2.4	
21	10.8	7.4	5.8	Weight 9.6 kgm.
22	12.5	18.7	10.7	Pants before and after injection. Discontinued
23	24.2	63.3	10.6	Pants violently after injection. Retches but does not vomit. Weight 10.2 kgm.
24	7.2	9.7	8.4	
25	17.4	5.7	5.7	

TABLE 7—*Concluded*

DATE	I	II	III	REMARKS
May				
26	20.4	13.9	11.4	Weight 10.6 kgm. Pants before and after injection
27	20.1	11.8	11.6	Vomits after injection
28	15.7	24.4	10.7	
29	21.9	32.4	15.3	Pants violently after injection
30	17.6	16.7	11.6	Pants before and after injection
31	20.8	13.1	12.2	
June				
1	8.9	17.1	15.3	
2	6.5	32.5	12.1	Pants after injection
3	7.6	10.1	9.0	
4	7.3	12.3	9.3	Vomits after injection
5	4.6	10.9	9.6	
6	7.6	9.5	8.8	
7	4.8	11.2	11.2	Retches after injection
8	7.4	4.7	4.7	
9	8.2	15.4	15.4	Pants, whines and vomits after injection
10	8.1	12.1	11.0	Pants after injection
11	7.9	12.6	12.5	Pants and vomits after injection
12	7.7	15.8	11.9	
13	8.2			Weight 12 kgm. Morphine injections discontinued
14	3.8			
15	1.6			
16	2.8			
17	2.3			
18	2.3			
19	2.1			
20	0.0			
21	0.7			

Tests made in the afternoon. Under I are given the quantities of saliva in cubic centimeters secreted in 15 minutes before morphine was injected. Under II are tabulated the quantities of saliva in cubic centimeters secreted after the morphine injection, and under III those portions of the latter which were secreted in the first 15 minutes after the injection.

and in two days the secretion rose to 11.3 cc. The data are plotted in figure 5, and it will be noticed that the secretion rose long before the weight of the animal returned to normal. A similar experiment performed on the second dog, "Goldie," produced the same picture of a rather slowly developing conditioned reflex, resulting in a maximum secretion of 6 to 7 cc. in 15 minutes, then suddenly beginning to fail (table 7). When the dog secreted only 1.1 cc. (May 19), starvation was discontinued, the morphine injections being kept up, and four days later the dog secreted 24.2 cc. in 15 minutes.

We next starved "Mother," easily developing the reflex in her, as this was her third series. She was very well fed, and the reflex rose to a considerable height. We then discontinued the morphine, continuing the starvation, and the reflex was promptly extinguished (table 5). On June 23, after she had been starving for 17 days, we started a new series of morphine injections, continuing the starvation. The reflex developed to some extent and then began to fail, almost exactly duplicating the picture obtained in "Hobo" and "Goldie." Figure 6 shows the immediate and striking improvement of the reflex on realimentation. In all four dogs studied starvation had a very destructive effect upon the development and course of the salivary conditioned reflex.

Withdrawal of water and food was a much more powerful depressant of the conditioned reflex than starvation alone. On one occasion we deprived "Mother" of water as well as of food for two days (July 25–27), at a time when her salivary conditioned reflex was at its height. On July 25, the last normal day, she secreted 42.7 cc. in 30 minutes. On July 26 she secreted only 21.8 cc., and on July 27, 10.3 cc., having lost 0.9 kgm. in these two days. She was fed and watered on July 27, and on the following day she secreted 37.2 cc., and on July 29, 53.1 cc. in 30 minutes.

On some occasions we allowed our dogs to remain in the stock for a period of time longer than usual, in order to see if they would continue to secrete saliva. We found that to be invariably the case. "Brindle" secreted 44.7 cc. in one hour on January 20, and when left in the stock for another hour, he secreted 40.5 cc. more. From "Tramp" we collected 29.5 cc. in 30 minutes of the regular run, and 22.8 cc. when he was left in the stock for another 30 minutes. "Mother" was usually placed in the stock for 30 minutes before giving her morphine, but on two occasions she was allowed to remain for one hour. On one of these occasions (May 1) we collected 45.3 cc. in the first half-hour and 43.3 cc. in the second; on the other occasion (July 17), the corresponding volumes were 50.8 cc. and 42.4 cc. The latter quantity of saliva, 93.2 cc., was the largest we ever obtained from the fistula of any dog in one hour.

The saliva obtained from the fistula was only a portion of the total amount of saliva secreted conditionally. The rest of the saliva, except the fraction which may have been swallowed, dripped from the mouth, when the reflex was in the early stages of development, or actually poured, when the reflex was fully established, as had already been observed by Collins and Tatum. In a pan placed directly under the dog's mouth we collected the drippings occasionally, to compare the volume of saliva that came from the fistula with the secretion of the remaining glands. In the case of "Rusty" we once collected 76 cc. of drippings in one hour, when for the same hour the secretion from the fistula amounted to 35.9 cc. On the

following day, in the same animal we got 77 cc. from the mouth as compared with 32.9 cc. from the fistula. "Tramp" secreted 40.1 cc. from the fistula (Apr. 15) and 140 cc. were collected in the pan under his mouth; on the following day the corresponding volumes of saliva were 28.1 cc. and 76 cc. On the two occasions when we ran "Mother" for a whole hour, instead of 30 minutes, we collected the drippings from the mouth and found that on the first occasion, when the secretion from the fistula was 88.6 cc., the volume of the drippings was 291 cc., and on the second occasion, when she secreted 93.2 cc. from the fistula, the drippings amounted to 225 cc. The volume of saliva collected from the fistula varied from about one-third to about one-fourth of the total volume of saliva collected, in different dogs, or in the same dog on different occasions, probably depending on the amount of saliva swallowed and on the evaporation from the open pan during the collection.

The largest quantity of saliva collected in one hour from both fistula and mouth was 380 cc., obtained from "Mother" on May 1. Such a loss of water, we thought, should affect the concentration of the blood. We observed, as did Collins and Tatum, that at times, after they secreted

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Fig. 6. The development of the conditioned reflex in "Mother" during starvation, showing a deterioration of the reflex in spite of continued daily injections of morphine, and the effect of re-alimentation (arrow) on the further development of the reflex. The data in the abscissa represent successive days. For numerical data see table 5.

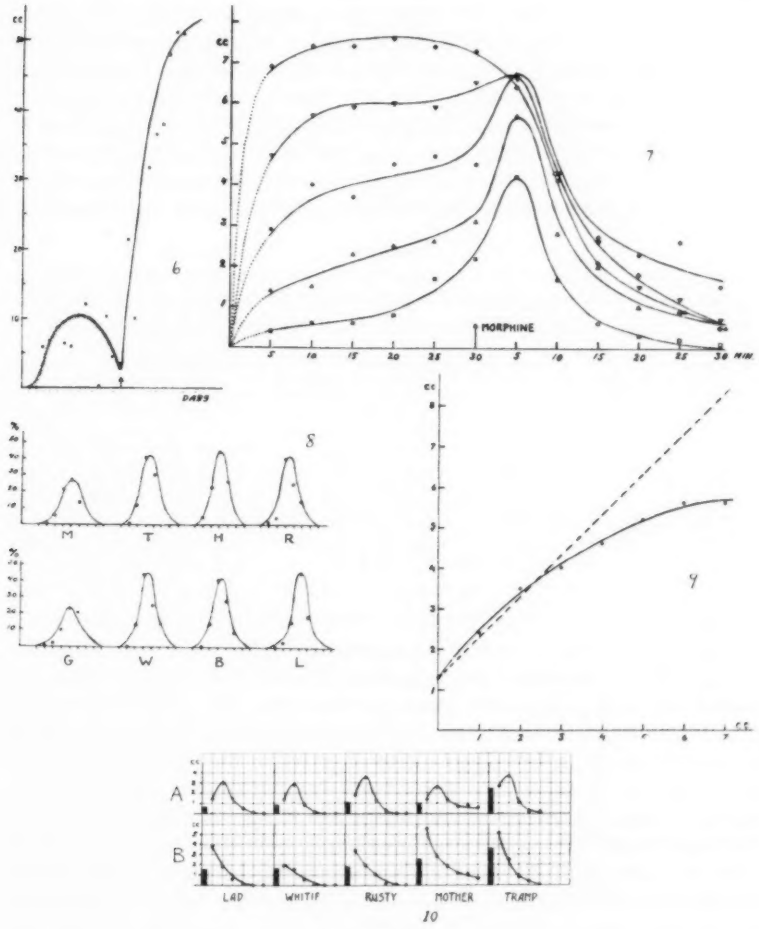
Fig. 7. Curves showing the average rate of secretion of saliva by "Mother" in successive 5-minute intervals before and after the injection of morphine, showing an increasing rate of secretion throughout the pre-morphine period when the total quantity of saliva secreted was small, and a practically uniform rate when the total quantity of saliva secreted was high. The rate of secretion in the first 5-minute period after the injection is higher, equal to, or lower than the rate for the last 5 minutes before the injection depending upon the magnitude of the latter. Decrease in the secretion rate for successive 5-minute intervals after the injection is shown. For data see table 8.

Fig. 8. The percentage (frequency) distribution curves of the duration of the secretion of saliva by each of the eight dogs after the injection of morphine. The divisions on the axis of abscissae correspond to 5-minute intervals after the injection. The curves all resemble probability curves, with the mode between 15 and 20 minutes. For figures see table 9.

Fig. 9. The correlation between the rate of secretion of saliva in the last 5-minute interval before the injections of morphine and the average rate for the first 5 minutes after the injection. The straight interrupted line shows the curve which should prevail if the action of morphine were purely additive. For numerical data see table 10.

Fig. 10. Curves showing the average secretion rate for successive 5-minute intervals in five dogs. *A*, when the secretion rate for the second 5-minute interval was greater than for the first, and *B*, when the opposite was true. The columns show the average secretion rate for the last 5-minutes before the injection in the corresponding classes. It will be noticed that the columns in *B* are almost twice as high as in *A*. The actual figures are given in table 11.

large quantities of saliva, the animals drank water freely. We therefore drew blood from some of our dogs, before and after they secreted saliva, and could detect no variation in the percentage of blood corpuscles upon centrifugation of the blood. When "Mother" secreted 380 cc. of saliva in one hour her blood corpuscle percentage changed from 48 to 49. In



Figs. 6–10

"Whitie" the percentage of blood corpuscles increased from 42 to 44 when we collected 93.8 cc. of saliva from the fistula alone. Similar results were obtained in other dogs. Water was lost from the body in large quantities, but the concentration of the blood, insofar as may be judged from hematocrit readings, remained unchanged. Water probably migrated from the tissues into the circulating blood.

The saliva secreted from the fistula was measured every 5 minutes, and this enabled us to determine the rate at which it was being secreted during the test. We found that the rate of secretion varied, especially during the development of the conditioned reflex. In the early stages, when the total amount secreted conditionally was only 1 or 2 cc., most or all of it was secreted in the latter part of the period the animal spent in the stock before it received morphine. Later on the dogs secreted throughout the whole pre-morphine period, but progressively more in succeeding

TABLE 8

The average rate of secretion of saliva by "Mother" in successive 5-minute intervals (indicated by the Roman numerals) before and after the injection of morphine on the basis of the total quantity of saliva secreted before the injection of the morphine

TOTAL SECRETED BEFORE INJECTION	NUMBER OF CASES	AVERAGE RATE OF SECRETION BEFORE INJECTION						NUMBER OF CASES	AVERAGE RATE OF SECRETION AFTER INJECTION					
		I	II	III	IV	V	VI		I	II	III	IV	V	VI
cc.		*cc.*	*cc.*	*cc.*	*cc.*	*cc.*	*cc.*		*cc.*	*cc.*	*cc.*	*cc.*	*cc.*	*cc.*
0–10	12	0.4	0.6	0.6	0.8	1.7	2.2	10	4.2	1.7	0.6	0.3	0.2	0.1
10–20	9	1.4	1.5	2.3	2.5	2.6	3.1	7	5.7	2.8	2.0	1.0	0.9	0.5
20–30	19	2.9	4.0	3.7	4.5	4.7	4.5	12	6.7	4.1	2.7	2.3	2.6	1.5
30–40	15	4.7	5.7	5.9	6.0	5.9	6.5	12	6.6	4.3	2.6	1.5	1.2	0.7
40–50	9	6.9	7.4	7.4	7.6	7.4	7.3	7	6.4	4.3	2.1	1.8	0.9	0.5

5-minute intervals. Finally when the conditioned reflex was fully established the rate of secretion was practically the same for the whole test period. "Mother's" average rates of secretion for successive 5-minute intervals, when the total secretion for the whole 30-minute period was large or small, are given in table 8 and are represented graphically in figure 7. When the total secretion for 30 minutes was 10 cc. or less, the 5-minute rate rose from 0.4 cc. in the first interval to 2.2 cc. in the last, but when the total output of saliva was from 40 to 50 cc. there was practically no variation in the amounts collected in successive 5 minute intervals. The results obtained on other dogs resembled those obtained on "Mother," except that in some dogs, notably "Tramp," the average rate of secretion was greater for the first 5-minute interval than for the second, imparting a secondary peak to the curve. The largest quantity of saliva collected from the fistula of any dog in 5 minutes was 11.2 cc., in the case of "Goldie," when her conditioned secretion flared

up on realimentation after fasting. But secretion rates of 9 to 10 cc. in 5 minutes were not uncommon. The whole question will be discussed below in connection with the rates of secretion that prevailed after the injection of morphine.

The conditioned stimulus, as already stated, was the set of conditions that accompanied the dog's standing in the stock, and this stimulus continued as long as the dog was in the stock, preceding the injection of morphine which acted as the unconditioned stimulus for the salivary secretion. The conditioned stimulus contained visual elements, since the dog could see the sides of the stock, and tactile elements, the contact of the skin of the neck with the stock and the contact of the lower jaw with the string and with the thistle tube. To see how important was the part played by the visual elements we blindfolded two dogs, and placed them in the stock in that condition. They could receive only tactile stimuli,

TABLE 9

The duration of the secretion of saliva after the injection of morphine tabulated according to the percentage of cases in which the secretion continued for one or more 5-minute periods

DOG	PERCENTAGE OF CASES IN WHICH SECRETION CONTINUED					
	5 minutes	10 minutes	15 minutes	20 minutes	25 minutes	30 minutes
Mother	0	5	21	*27*	13	10
Tramp	0	12	*41*	31		
Hobo	5	23	*45*	27		
Rusty	2	4	*41*	25	16	
Goldie	0	2	10	*23*	21	
Whitie	0	13	*44*	26	14	
Brindle	0	4	*42*	28	9	
Lad	0	3	16	*47*	19	

and while the conditioned reflex was elicited, it was considerably depressed. "Whitie," on the day preceding the blindfolding, secreted 19.7 cc., on the day he was blindfolded (Jan. 24) 6.6 cc., and on the day following 12.1 cc. "Brindle" under similar circumstances secreted 33.3 cc., 16.9 cc. (Jan. 25) and 42.9 cc.

Up to now we described only the events that occurred before the injection of morphine. But we have gathered considerable data on the behavior of the animals after the injection. The actual insertion of the needle and the injection was taken very good-naturedly by the dogs after they got used to it. The dose of morphine given did not always cause vomiting or even retching. All the animals were run on an empty stomach, and that may account for the infrequency of vomiting. When they did vomit, it was always 5 or 6 minutes after the injection. At any rate, the dogs almost invariably showed symptoms of excitement or even

distress, panting, whining, etc. It was clear that they were markedly nauseated. This was soon followed by depression and muscular relaxation. The dog allowed its body to hang from the stock, became very somnolent, and after a variable length of time the secretion of saliva stopped. In the majority of cases the secretion stopped in 15 or 20 minutes, but occasionally it continued for a much longer time. "Goldie" and "Mother" were often taken out of the stock 30 minutes after the injection of morphine, while they were still secreting very well. The record for after-injection secretion is held by "Goldie" who frequently continued to secrete for an hour or two. In one case (May 23) she secreted 63.3 cc. from the fistula in 2½ hours after the injection, and was still going strong when the collection of saliva was discontinued. For each dog we determined the number of cases in which it secreted for 5, 10, 15, 20, 25 and 30 minutes after the injection, and we tabulated these

TABLE 10

The correlation between the rate of secretion of saliva in the last five minutes before the injection of morphine and the corresponding average rate of secretion in the first five minutes after the injection of morphine, based on the data obtained from all the dogs

NUMBER OF CASES	RATE OF SECRETION IN LAST 5 MINUTES BEFORE INJECTION	RATE OF SECRETION IN FIRST 5 MINUTES AFTER INJECTION
	cc.	cc.
26	0.0	1.3
33	0.0-1.0	2.4
74	1.1-2.0	3.5
56	2.1-3.0	4.0
39	3.1-4.0	4.6
22	4.1-5.0	5.2
18	5.1-6.0	5.6
15	6.1-7.0	5.6

numbers in percentages of the total number of tests. The figures are given in table 9, and one will notice that the post-morphine secretion lasted most frequently for 15 minutes in five dogs and for 20 minutes in three, the latter including "Mother" and "Goldie." The percentage distribution curves for the duration of the post-morphine secretion are shown in figure 8, and they look like probability curves with the modes between 15 and 20 minutes.

In a general way the secretion after morphine showed a gradual diminution in each successive 5-minute period and died out at the end of 15 or 20 minutes. The actual magnitude of the secretion depended upon the rate of conditioned salivation that prevailed before the injection of morphine. The figures collected in table 8 show that up to a certain limit the greater the total secretion before the injection, the greater the rate of secretion after it. In table 10 we correlated the average rate of secre-

tion for the first 5 minutes after the injection with the rate of secretion in the last 5-minute interval before the injection. In the early stages of development of the reflex, when the animal secreted no saliva before the injection of morphine, the average rate for the first 5-minute interval after the injection was 1.3 cc.; when the pre-injection rate was 1 cc. or less, that after the injection was 2.4 cc.; and so on up the scale, until when the animals secreted from 5.1 to 6 cc. in the last 5 minutes before the injection, their average secretion for the first 5 minutes after the injection was only 5.6 cc., the same average post-injection rate prevailing when the pre-morphine rate was from 6.1 to 7 cc. These data are given in graphic form in figure 9, the curve showing a negative acceleration.

TABLE 11

The average rate of secretion of saliva in successive 5-minute periods (indicated by Roman numerals) after the injection of morphine in comparison with the average rate of secretion for the last five minutes before the injection

DOG	NUMBER OF CASES	5 MINUTES BEFORE INJECTION	5 MINUTE PERIODS AFTER INJECTION					
			I	II	III	IV	V	VI
A. When the secretion during the first 5-minute period after the injection was less than during the second								
		cc.	*cc.*	*cc.*	*cc.*	*cc.*	*cc.*	*cc.*
Lad	17	0.7	1.4	3.0	1.4	0.5	0.1	
Whitie	29	0.8	1.3	2.8	0.8	0.2		
Rusty	22	1.1	1.8	3.5	1.3	0.3		
Mother	14	1.0	1.3	2.6	1.3	0.7	0.8	0.6
Tramp	13	2.4	2.6	3.7	1.1	0.2	0.2	
B. When the secretion during the first 5-minute period after the injection was greater than during the second								
Lad	19	1.5	3.8	1.8	0.6	0.2		
Whitie	62	1.6	1.9	1.5	0.5	0.1		
Rusty	28	1.8	3.3	1.9	1.0	0.2	0.1	
Mother	42	2.5	5.6	2.8	1.8	1.2	1.1	0.7
Tramp	44	3.6	5.1	2.5	0.8	0.3	0.1	

The rate of secretion for successive 5-minute intervals after the injection of morphine does not always vary in the same sense when the rates for the first and second periods are compared. In some cases the animals secreted more in the first than in the second 5-minute interval, and in other cases the reverse was true. We divided the figures for post-morphine secretion by each dog into two classes: *A*, when the secretion in the first 5-minute interval was smaller than in the second, and *B*, when the secretion for the first 5 minutes was greater than for the second. For each of these two classes we determined the average rate of secretion for the

last 5 minutes before the injection of morphine, and the average rate of secretion for each of the successive 5-minute intervals after the injection. The figures are presented in table 11 and are plotted in figure 10. It will be seen that the gradual decrease in the average rate of secretion from the second 5-minute interval on is the rule for both classes, but that the average rate of secretion for the last 5-minutes before the injection in class *A* is in each dog only about one-half of what it is in class *B*. There is then a definite connection between the form of the curve of the post-morphine, or unconditioned, secretion and the average rate of secretion for the last 5-minute interval before the injection, aside from the dependence of the secretion for the first five minutes after the injection upon the rate of secretion in the last 5 minutes before the injection.

We have indicated that when the dogs secreted saliva they gave many indications of being nauseated, but we wanted to determine if any of the post-morphine secretion of saliva could be ascribed to direct action of morphine. Although a priori one could not expect morphine to act on nerve endings in the salivary glands, the possibility of its stimulating the salivatory nucleus in the medulla had to be excluded by actual experiment. We anesthetized dogs by means of barbital and inserted cannulae into the ducts of the submaxillary glands. Morphine injected subcutaneously and intravenously into these animals failed to produce any flow of saliva, even when the doses were large. We always followed up the morphine injections with the administration of small doses of pilocarpine. This drug invariably produced a copious flow of saliva, indicating that the glands were in a condition to secrete.

Discussion. We just pointed out that morphine did not act directly on the salivatory nucleus in the medulla. When injected into a dog, it calls forth a secretion of saliva, among many other things. The symptom complex, of which salivation is a part, is called nausea. But what is nausea? Is it only a feeling? Can it be separated from the motor and secretory phenomena that accompany it? These questions are difficult to answer. We are inclined to look upon nausea as a sensory-motor-secretory complex of which secretion of saliva is one component. It just happens that it is the component we can measure, and we think that its magnitude may be taken as an indication of the degree of nausea developed in the animal. The same rule applies to other conditioned reflexes that are a part of a complex response. If a dog with a salivary fistula is shown some food, saliva can be seen to trickle from the fistula. The magnitude of the secretion is a measure of the desire of the dog for food. If there is no desire for food, the animal does not secrete saliva. Pavlov (1910) states that "a psychic response is only obtained when the animal is hungry." So will morphine produce a secretion of saliva only if it nauseates the animal. We feel certain that if we could measure some

of the other secretions that accompany nausea, we would find them to run parallel with the secretion of saliva. Of course, not all conditioned reflexes are parts of complex responses. If one throws light into the eye, he produces a reflex constriction of the pupil. By repeatedly applying an auditory stimulus at the same time as the light stimulus, one can develop a conditioned pupillary reflex to sound. Here is an example of a conditioned reflex that is fairly simple (perhaps not entirely free from accompanying responses), the unconditioned stimulus acting directly on the end-organs in the retina and producing a limited response. But morphine when injected subcutaneously does not act upon some end-organs exclusively, nor upon a definite single center in the medulla. It acts upon many centers in the nervous system, producing, among other effects, nausea, of which salivation is a part. The experience of Collins and Tatum, as well as our own, convinces us that the dogs were nauseated during the conditioned salivation that preceded the injection of morphine. The panting and whining and the general manifestations of distress indicated that the dogs were nauseated. But the weightiest argument in support of this contention is that the dogs occasionally *vomited* before they received the injection of morphine. The conditioned reflex we studied can be used as means of measuring the degree of nausea in the dog. We want the reader to bear in mind that what we say about the development and extinction of the conditioned salivary reflex applies, we believe, with equal force to the development and extinction of nausea, but having made our position clear, we shall hereafter speak of the salivary reflex as such, that being the one we actually studied quantitatively.

There are two important features in the method of developing the conditioned reflex that we employed, in which it differs from methods used by others. First, we applied only one conditioned and one unconditioned stimulus per day, whereas most investigators applied several combinations of the two during one test. Second, instead of a simple conditioned stimulus, such as the ringing of a bell, we used a set of conditions that had various elements in it. That each of these elements counted in the elicitation of the conditioned reflex was shown by the results of our blindfolding tests, where the absence of the visual elements in the set of conditions resulted in a diminution of the conditioned response. We kept up the action of our conditioned stimulus for a long period of time before applying the unconditioned one (longer than had ever been done before, to our knowledge), and yet the response was not "delayed" as one might expect it would be. Our first dog was left in the stock for two hours prior to the administration of morphine, but he never learned to postpone his salivation until such time as he knew he would receive something that always made him nauseated. When the reflex was fully developed, the dog kept up a constant flow of saliva throughout the period

of waiting for the injection of morphine. The dog did not become somnolent, and so we were unable to accomplish the object of our research. But this is not in direct contradiction with the requirements of Pavlov's theory of sleep. Pavlov maintains that sleep is produced by local cortical inhibition which spreads and produces a general inhibition, which is the cause of sleep. Since we obtained no inhibition of the conditioned response through delaying the application of the unconditioned stimulus, we could hardly expect sleep to result. However, our results differ from those obtained by the workers in Pavlov's laboratory in that no delayed conditioned reflex was developed at all. Indeed, just the opposite was true. In the early stages of development of the reflex the animals would begin to secrete in the latter part of the test period, but as the reflex became more established, the secretion began to pour out earlier, at a gradually increasing rate of flow, until in the fully established reflex the flow reached a maximum value as soon as the animals were placed in the stock and remained constant for the whole pre-morphine period. Nor did our dogs stop secreting saliva if, at the end of the customary test period in the stock, they did not receive morphine. They only slowed down a little. In other words, when the conditioned reflex was fully developed, the dogs became nauseated and secreted saliva as soon as they were placed in the stock, and they continued to secrete saliva as long as they were kept in the stock, or until the depression that sets in some time after the injection of morphine abolished the nausea and checked the salivation.

The fact that a conditioned reflex develops gradually until it reaches a certain maximum, which may be called its full strength under the conditions of the experiment, has not been universally accepted. Lashley (1924) who had done some work on learning, holds that it is fully integrated when it appears for the first time. According to him, "improvement can be expressed only in terms of the proportion of times when the associated stimulus succeeds or fails to elicit it." True, in the same place he says that "the 'all or nothing' law governs the appearance of some conditioned reflexes." So it is not clear whether he intends to make his first statement as general as it seems to be. But Herrick (1926), in his recently published dissertation on *Brains* quotes Lashley with the following comment (p. 65):

> In another place, Lashley says, "it is characteristic that the conditioned reflex is not built up gradually, but is fully integrated when it first appears." Of course, the last statement must be true, for this type of learning does not involve the gradual formation of new elementary behavior patterns but only the doing in unfamiliar situations of perfectly familiar things for which definite neural mechanisms are already laid down in innate or habitual organization. When an auditory stimulus which does not ordinarily produce salivation is diverted to the salivary path, a fully

integrated salivation follows as soon as the proper central connection is made, for the salivatory mechanism is preformed and ready to operate perfectly as soon as it is activated from any source.

While Herrick is trying to show, on a theoretical basis, why Lashley's statement must be true, our results, on an experimental basis, show that it is false. The conditioned reflex, at least the one we studied, is not fully integrated when it first appears, but is built up gradually, and according to a very definite law. Anrep's work on pitch discrimination in the dog (1920) shows clearly the gradual development of the salivary conditioned reflex to sound. He tabulated the number of times the conditioned and unconditioned stimuli must be applied together in order to develop the reflex fully. After 10 combinations one of his dogs secreted 6 drops of saliva in 30 seconds in response to the conditioned stimulus alone, after 20 combinations, 20 drops, after 30, 60 drops, and that was as much as it ever secreted conditionally afterward. In another dog it also took 30 combinations of the conditioned and the unconditioned stimuli to build up the reflex to its maximal strength. We shall refer to Anrep's work again when we speak of the reëstablishment of the conditioned reflex.

As we already indicated, the curve of development of our conditioned reflex was peculiar in that it showed a positive acceleration at first, and a negative acceleration afterward. We recalled in this connection Mathews' description of the properties of linseed oil which, according to him, manifests changes resembling the processes of learning, forgetting and relearning. In his *Physiological Chemistry* (1925) he has a curve showing the rate of absorption of oxygen by linseed oil, and it is an S-curve. The process is autocatalytic, the product of the reaction acting as a catalytic agent. Mathews comments upon "the presence in the cephalin of the brain of very unsaturated fatty acids of the type of linoleic acid," and adds that "if it were possible that an impulse coming into certain cells caused in those cells the formation of a persistent autocatalytic, intermediary oxidation product, a physical basis of memory might be given."

Robertson, in his *Biochemistry* (1920), goes farther than Mathews in that he shows that the process of memorizing nonsense syllables by the Ebbinghaus method obeys the law for the course of autocatalyzed monomolecular reactions. The reader is referred to the textbook for the derivation of the autocatalytic formula, which is as follows:

$$\log \frac{x}{a - x} = k\, a\, (t - t_1)$$

In this formula a is the total amount of material to be transformed by the reaction, x, the amount of material that should be transformed in time t, k, a constant specific for the particular reaction under consideration, and t_1, the time at which the reaction is half completed, the center of the curve.

We found that the results obtained on our dogs fit fairly well into such a curve, the deviation of the observed from the calculated ones being within the limits of experimental error. Table 12 shows the figures for the salivation obtained during the development of the conditioned reflex in "Mother." The quantity of saliva secreted conditionally at each test corresponds to the amount of material transformed in the cortical centers in the process of establishment or learning of the reflex (x), a is the maximum quantity of saliva the dog will secrete in 30 minutes when the reflex is fully developed (we took it to be 48 cc. which is close to the actual maximum), t_1 for "Mother" is between the eighth and ninth tests, and k is 0.0038.

TABLE 12

A comparison of the experimentally obtained figures for the secretion of saliva by "Mother" during the development of the conditioned reflex with the calculated figures which should prevail A, if the development followed the curve for monomolecular autocatalyzed reactions, and B, if it followed the Gamma function curve

X OBSERVED	$A.$ X CALCULATED AUTOCATALYTIC	DEVIATION	$B.$ X CALCULATED GAMMA FUNCTION	DEVIATION
0.4	2.0	1.6	1.4	1.0
0.4	3.0	2.6	2.4	2.0
2.9	4.3	1.4	3.8	0.9
0.6	6.3	5.7	6.2	5.6
8.1	9.0	0.9	9.1	1.0
21.9	12.4	9.5	12.5	9.4
21.8	16.7	5.1	17.3	4.5
22.0	21.5	0.5	22.2	0.2
20.6	26.5	5.9	26.8	6.2
31.8	31.3	0.5	31.6	0.2
36.5	35.5	1.0	36.0	0.5
35.7	39.0	3.3	39.7	4.0
44.6	41.7	2.9	42.2	2.4
45.3	43.7	1.6	44.4	0.9
		A. D. = 3.0		A. D. = 2.8

At this point we want to say that in all our dogs we observed some fluctuations in the amount of saliva collected from day to day, both during the development of the reflex and when the latter was fully established. The observed values fall on each side of the theoretical curve. Our experimental conditions were by no means perfect. We do not agree with Anrep that the behavior of the experimenter himself affects the conditioned reflex, especially if the animal is accustomed to the presence of the observer at all times. In our work it often happened that the experimenter, while the test was in progress, had to leave the room, and on returning he always found that the animal secreted just as well (or

just as poorly), when it was left alone as when the observer was present. We do admit that there were a number of conditions that might have been controlled and were not. We showed, for instance, that lack of food, and especially of water, acts deleteriously upon the conditioned reflex. Our dogs were fed table scraps, and their diet was uniform neither in quality nor in quantity. At times they may have been short of water. What strikes us as remarkable is not that there were fluctuations in the conditioned secretion of saliva, but that in spite of imperfect experimental conditions the observed values corresponded so closely to the calculated ones.

We plotted Anrep's figures for the secretion of saliva during the development of the conditioned reflex, the number of trials in the abscissae and the drops of saliva per 30 seconds in the ordinates, and for each of his dogs we obtained a semblance of an S-curve. We are indebted to Dr. Elmer Culler for calling our attention to three psychological papers on the learning process, in which the S-curve characterizes the manner in which perfection was approached. In one of these, Chapman and Hills (1916) studied the curve of learning to use the typewriter. They studied two groups of students. The curve for students who had from 20 to 80 hours of practice showed a positive acceleration, whereas the curve for advanced students, who had practiced from 75 to 165 hours was characterized by a negative acceleration. In the second paper Thurstone (1919) reported some data on acquiring skill in typewriting, and if his figures for the speed of typing are plotted against the time, one obtains an S-curve. Most interesting of all is the third paper, a monograph on *The quantitative aspects of the evolution of concepts* by Hull (1920) where the author concludes that "the curve of the evolution of concepts begins with an initial period of positive acceleration which goes on to a maximum rate after which a period of negative acceleration sets in which continues to the end of the process." We may add that Doctor Culler considers all these S-curves as Phi-function of Gamma (probability integral) curves, and he has kindly agreed to treat the figures we obtained on "Mother" from this standpoint. The S-curve which he has drawn for the process of development of the conditioned reflex, on the basis of the Gamma function corresponds even closer to our observed values than the autocatalytic curve, but this may be due to a superior method of fitting that he employed. It can be seen from table 12 that the two sets of calculated values are very close to each other, and the two curves in figure 1, drawn from these figures, are almost identical. We have difficulty, however, in conceiving of the development of the conditioned reflex as a psychometric function, and it seems to us that an autocatalytic process is most probably the underlying cause responsible for the S-curves which characterize the phenomenon of learning by multiple repetition.

We do not advance our results as a proof of the autocatalytic nature of certain types of learning processes, but merely as another link in the slowly forming chain of evidence that these processes may be autocatalytic reactions. At least, they behave as if they were such reactions. It is doubtful if we will ever be able to test this hypothesis chemically, and for the present, at any rate, we must depend upon circumstantial evidence. It may be pointed out that the other learning processes we referred to all involve an element of effort, which makes them more complex. In the case of our dogs we have a development of a conditioned reflex (or learning to salivate when placed in the stock) by repetition of a combination of a conditioned stimulus and an unconditioned one, pure and simple.

The extinction of the conditioned reflex upon discontinuing the daily injections of morphine is no more sudden than its development. The curve of gradual abolition of the reflex differs, however, from the curve of establishment. In the manner in which they lost the reflex our dogs may be divided into two groups. The first group comprises "Whitie," "Mother" and "Rusty." In these dogs the reflex becomes extinguished very rapidly, and is generally completely gone 4 or 5 days after stopping the injections of morphine. The rate at which the reflex deteriorates is not the same from day to day. The figures for the salivary secretion plotted against the time show a rapid falling-off at first, then a slower approach to zero. The curves are concave upward, but as the direction of the curves is from above downward, they show a negative acceleration. The curves are almost perfect second degree parabolas, and table 13 shows how close the observed values are to the calculated ones. If the conditioned reflex is reëstablished, and then allowed to become extinguished, the curve of the second abolition is the same as that of the first one. Figure 2 contains one curve for "Rusty," two for "Whitie" and two for "Mother." All are alike. If one examines the observed values as plotted around the parabolas, one can notice that in all the curves but one the first two points are on the curve, whereas all the subsequent observed values are smaller than the calculated ones. It seems to us that this acceleration of the extinction of the conditioned reflex may be due to an element of learning affecting the process of forgetting, and in a positive sense. In other words, when the injections of morphine are discontinued, the dog gradually returns to normal, i.e., forgets to become nauseated when placed in the stock, but after a couple of days the dog also learns not to become nauseated, and that accounts for the quicker extinction of the reflex. This is a mere suggestion, and is advanced as a possibility. The second group of dogs, to which belong "Hobo," "Brindle" and "Tramp," show a much slower abolition of the conditioned reflex upon discontinuing the injections of morphine. It took these dogs from one to over two weeks to stop secreting saliva when placed in the stock (fig. 3).

In these dogs we witness a simple returning to normal, probably unaccompanied by the element of learning (in the sense of ideation) referred

TABLE 13

A comparison of the experimentally obtained figures for the secretion of saliva by "Whitie," "Mother" and "Rusty" on successive days during the abolition of the conditioned reflex with the calculated figures which should prevail if the curve of abolition of the reflex were a second degree parabola

WHITIE I; $a = 92.3$

X	OBSERVED Y	CALCULATED Y	DEVIATION
1	92.3	92.3	0.0
2	29.6	23.1	6.7
3	2.9	10.3	7.4
4	3.2	5.8	2.6
5	1.8	3.7	1.9
6	1.5	2.6	1.1
7	0.0	1.9	1.9
			A. D. = 3.1

WHITIE II; $a = 18.1$

X	OBSERVED Y	CALCULATED Y	DEVIATION
1	18.1	18.1	0 0
2	5.1	4.5	0.6
3	1.5	2.0	0 5
4	1.0	1.1	0.1
5	0.0	0.7	0.7
			A. D. = 0 4

MOTHER I; $a = 45.3$

X	OBSERVED Y	CALCULATED Y	DEVIATION
1	45.3	45.3	0.0
2	11.0	11.3	0.3
3	3.1	5.0	1.9
4	0.0	2.8	2.8
			A. D. = 1.3

MOTHER II; $a = 53.1$

X	OBSERVED Y	CALCULATED Y	DEVIATION
1	53.1	53.1	0.0
2	29.2	13.3	15.9
3	0.5	5.9	5.4
4	0.0	3.3	3.3
			A. D. = 6 2

RUSTY; $a = 32.9$

X	OBSERVED Y	CALCULATED Y	DEVIATION
1	32.9	32.9	0.0
2	8.4	8 2	0.2
3	0.5	3.7	3.2
4	0.0	2.1	2.1
			A. D. = 1.4

$y = \frac{a}{x^2}$, where y is the quantity of saliva secreted on any day, a, a constant, the quantity of saliva secreted on the day the injections of morphine were stopped, and x, the number of days that passed since the last injection of morphine.

to in connection with the behavior of the first group of dogs. It was our impression that the dogs of the second group were more stupid than the

dogs of the first group. But instead of dwelling upon the differences in the manner in which the reflex is extinguished in the two groups, we should like to emphasize the similarities. In *all* the dogs the curves of extinction of the conditioned reflex show a negative acceleration. The rate at which the response is forgotten is greatest in the beginning, and then gradually decreases. In the second group the curve tends to approach the X axis asymptotically.

We turn once more to Robertson's *Biochemistry* and find that the curve for forgetting the Ebbinghaus nonsense syllables differs from the curve of learning in exactly the same way in which the curve of extinction of the conditioned reflex differs from the curve of establishment. Robertson plotted his values in terms of the fraction forgotten, while we plotted ours in terms of the fraction retained. Therefore his curve looks like ours inverted. He compares the curve of forgetting with a "curve which expresses the rate of issuance of a colloid from a colloidal into a fluid menstruum," say, the rate at which suspended casein is dissolved by potassium hydroxide, and finds them very much alike. He does not think that forgetting involves a chemical reaction, but rather that it may be due to the "washing out of a colloidal substance, which forms the memory-trace, by the circulating fluids." As the complete extraction of a colloid from a colloidal menstruum by an external liquid may take years and years, this hypothesis "would explain at once the rapidity of the initial stages of forgetting and the extraordinary persistence of the last traces of the memory-deposit."

The conditioned reflex once extinguished may be reëstablished by a new period of training. The length of time it takes to redevelop the reflex is materially less than the time it took to develop it for the first time. The curve of reëstablishment differs from the curve of establishment in that the initial portion of the S-curve is missing, the acceleration being zero at first, then becoming negative. It looks as if the curve started at a higher point than it did the first time. The figures given by Anrep demonstrate the same phenomenon. In each of the two dogs in whom he established the conditioned reflex for the first time, it took 30 combinations of the conditioned and unconditioned stimuli for the secretory response to attain its maximal value. In two other dogs in whom the reflex was reëstablished it required 16 combinations in the first and 11 in the second for the reflex to become fully developed. As regards the nonsense syllables, it is well known that it takes less time to relearn them, after one has completely forgotten them, than it takes to learn them for the first time. The curve of forgetting the nonsense syllables is constructed by determining the time it takes to relearn them after various lapses of time. We should distinguish between the apparent zero of performance and the physiological level of remembering which may have a

definite magnitude. This magnitude is of no value when it comes to performance, but is of great value in relearning. Two persons, of which one never heard of a certain poem and the other had known but completely forgotten it, are at exactly the same apparent level insofar as their ability to recite the poem is concerned, but they are at quite different levels when it comes to learning to recite it. Likewise when two dogs of which one had not been used before and the other had the salivary reflex developed and abolished are placed in the stock neither of them will secrete any saliva, and to the casual observer they will appear to be in the same physiological condition insofar as conditioned salivation is concerned. But if one tried to develop the reflex in these two dogs, one would find that the first dog will start at zero and go through the entire S-curve in the process of acquiring the conditioned reflex, while the second will not linger along the axis of abscissae, but will start climbing at once and reach the top much sooner.

It is evident that the establishment, extinction and reëstablishment of the conditioned salivary reflex resemble closely learning, forgetting and relearning Ebbinghaus nonsense syllables, to limit ourselves to a process of learning in man that involves simple multiple repetition.

The conditioned reflex may be established even if the application of the combination of the conditioned and the unconditioned stimuli is alternated with the application of the conditioned stimulus alone, as evidenced by the results obtained on "Mother" and "Lad." The effect produced by the combination is not completely neutralized by the omission of the unconditioned stimulus the following day, but the process of development of the conditioned reflex is slowed up considerably, and the reflex is imperfectly established.

Froloff (1925), in Pavlov's laboratory, studied the effect of chronic undernutrition on the salivary conditioned reflex. The study was made during the famine period when they could not get food for the animals. The dog that Froloff used weighed 20 kgm. originally, but lost 7.6 kgm. in five months. Food was the unconditioned stimulus. The animal had a fistula of the parotid gland, and on establishing the reflex Froloff obtained 5 drops of saliva in 30 seconds after 18 tests. The rate of secretion dropped to 2 drops per 30 seconds at the end of the 102nd test, and a little later no conditioned response could be elicited. But even then the dog responded well to the sight and smell of food, that is, he preserved the "natural" conditioned salivary reflex, to use Pavlov's terminology. In the conditioned reflex we studied food played no part whatever, and we thought that acute inanition might be tried to advantage in bringing out the effects of undernutrition, should there be any. Experiment showed that the fully established conditioned reflex deteriorated rapidly when the animal was starved (fig. 4). In attempting to establish the condi-

tioned reflex in starving animals we found that the initial portion of the curve of development did not differ from normal. In "Hobo" and "Coldie," in whom the reflex was established for the first time, the curve lingers along the axis of abscissae, as an S-curve should, and then begins to shoot upward (fig. 5); in "Mother," who had this reflex established in her three times before, the curve turns up at once (fig. 6). Apparently the undernutrition in the early stages was not marked enough to affect the curve of establishment of the reflex But later on, in all three dogs, the curve instead of continuing to go up, takes a sudden turn downward toward extinction. The effect of realimentation upon the curve of establishment of the reflex was immediate and very striking, and this made the problem more complicated, because the rapid rise in the secretion rate came long before the weight of the animal returned to normal. We were wondering whether the deterioration of the reflex in starvation was due to gradual atrophy of the salivary glands, or to some change in the central nervous system. The fact that the salivary glands are not markedly reduced in size in starvation, as well as the rapid development of the reflex upon realimentation, spoke against a peripheral cause. To test this hypothesis, however, we determined the effect of a given dose of pilocarpine, which acts peripherally, on a dog with a salivary fistula, in different stages of starvation. The results were not very conclusive, but were negative in the main. We could detect no real difference in the amount of secretion produced by a given dose of pilocarpine as starvation progressed, nor any increase upon realimentation. We expect to repeat these experiments more extensively in the future, and will make no conclusion at present. If it can be proven that the effect of starvation on the development of the conditioned reflex is central in origin, it should throw some light on the cause of the poor learning ability of undernourished children.

Dogs are known to drink little water while they are being starved. Doctor Carlson suggested that it is possibly "under-drinking" that is the cause of the failure of the conditioned reflex in starvation. The marked and immediate effect of withdrawal of water from "Mother" on the rate of her conditioned secretion indicates that that may very well be the cause. It can be further tested by starving dogs and giving them a definite quantity of water daily by means of a stomach tube, and we intend to do it shortly.

The effect of injecting morphine consisted of stimulation followed by profound depression. During the period of stimulation the animals were nauseated. If we may judge of the length of the period of stimulation by the duration of the nausea, and the duration of the latter by that of salivation, we would say that it lasted from 5 minutes to more than two hours, but more frequently from 15 to 20 minutes (table 9 and fig. 8). But a study of the rate of secretion of saliva for successive 5-minute intervals

following the injection of morphine revealed that the point at which depression definitely sets in is connected with the degree of development of the conditioned reflex, that is, with the condition of the animal prior to injection. In the first place, we tried to correlate the rate of secretion that prevailed in the first 5-minute interval after the injection with that for the last 5 minutes before the injection. We pooled the figures for the secretion rate in the last 5 minutes before the injection obtained on all our animals, and we divided them into several classes, according to whether the rate was 0, or less than 1 cc., or from 1.1 to 2 cc., etc. Opposite each figure in every one of these classes we placed the figure for the quantity of saliva secreted by the dog in the first 5 minutes after the injection on that day. Then we obtained the average rate for the first 5 minutes after the injection for each of the above classes, and the results of this correlation are given in table 10, and the values are plotted in figure 9. When the animals secreted no saliva in the last 5 minutes before the injection, they secreted on the average 1.3 cc. of saliva in the first 5-minute interval after the injection. If the secretion of this 1.3 cc. is caused by the direct action of the unconditioned stimulus, then it should add itself to the rate of secretion that prevailed before the injection of morphine, assuming, as we are justified in doing, that the animal would go on secreting at the same rate, if it did not receive an injection of morphine. That, we find, holds more or less true, when the secretion rate before the injection is low, but not as it increases. For instance, in those cases where the secretion rate for the last five minutes before the injection was from 6.1 to 7 cc., the secretion rate after the injection should have been from 7.4 to 8.3 cc., if the effect of morphine were purely additive, but as a matter of fact the secretion for the first five minutes after the injection in these cases amounted only to 5.6 cc. on the average. In other words, there may be a depression of the secretion, instead of a stimulation, even in the first 5 minutes after the injection. The greater the rate of secretion before the injection, the weaker the stimulation of the secretion after the injection up to a certain point, and the greater the depression in the first five minutes after the injection beyond that point. This is nicely corroborated by the figures for "Mother," collected in table 8 and plotted in figure 7. When the average rate of secretion for the last 5 minutes before the injection was 2.2 cc., that for the first 5 minutes after the injection was 4.2 cc., almost twice as much. When the figure for the last 5 minutes before the injection was 4.5 cc., the corresponding figure for the first 5 minutes after the injection was 6.7 cc., or only $1\frac{1}{2}$ times as much. Finally, when the animal secreted, on the average, 7.3 cc. before the injection, she secreted only 6.4 cc. after the injection, showing a depression, instead of a stimulation. It appears that morphine stimulates the secretion up to a certain point and then depresses it, but if that point had been reached or passed

through conditioned salivation before the injection of morphine, depression sets in at once, or at least within the first 5 minutes after the injection. This supposition is further confirmed by the nature of the post-injection secretion curves. In examining the figures for the secretion rates in successive 5-minute intervals after the injection we noticed that in certain cases the secretion was greater in the first post-injection interval than in the second, in other cases it was greater in the second than in the first. For each dog we grouped all the figures for post-morphine secretion into two classes according to whether the greater rate of secretion prevailed in the second or in the first 5-minute interval. We also determined the average rate of secretion for the last 5 minutes before the injection for each of the two classes. The figures given in table 11 and plotted in figure 10 show that the secretion rate for the last 5 minutes before the injection in class *A* (where the secretion in the first 5 minutes after the injection is less than in the second) was only one-half of what it was in class *B* (where the opposite is true). That means that when there was little secretion in the pre-morphine period, the stimulation of the secretion produced by morphine reached its peak only in the second 5-minute interval after the injection, and depression set in the *third*. In class *B* there is a higher rate of secretion in the first 5-minute interval after the injection than in either the last 5-minute interval before the injection or in the second 5-minute interval after the injection. Here the height of stimulation was reached in the first post-morphine 5-minute interval, and depression set in in the *second*. Then when the rate of secretion before the injection was still greater, say, from 6.1 to 7 cc. (table 10), there was secreted less saliva in the first 5-minute interval after the injection than in the last five minutes before the injection, which means that the depression set in in *first* 5-minute interval after the injection. This would explain the apparently paradoxical phenomenon that some dogs, when the conditioned reflex was fully developed, secreted saliva at a higher rate under the influence of the conditioned stimulus than as a result of the application of the unconditioned stimulus, the injection of morphine (table 8, last row of figures).

We are tempted to apply what we said about the interplay of stimulation and depression of the salivary secretion as produced by morphine to nausea itself, but we want to limit our analysis to the phenomenon we actually studied quantitatively, the secretion of saliva.

SUMMARY

1. By daily subcutaneous injections of morphine into dogs we were able to produce a conditioned salivary reflex, confirming Collins and Tatum.

2. The injections of morphine produce conditioned nausea, of which salivation is one component.

3. By continuing the conditioned stimulus for as long as two hours before applying the unconditioned one we were unable to produce a "delayed" conditioned reflex.

4. The conditioned reflex studied is not fully integrated when it first appears, but is built up gradually, confirming Anrep.

5. The rate at which saliva is secreted in successive 5-minute intervals before the injection increases with each 5 minutes in the early stages of development of the reflex, but is practically constant when the reflex is fully developed.

6. The total quantity of saliva secreted conditionally in the course of one hour by a 12 to 14 kilo dog may run as high as 300 to 400 cc.

7. The development of the salivary conditioned reflex follows an S-curve, showing first a positive acceleration, then a negative acceleration, and resembling the curve for autocatalized monomolecular reactions.

8. The extinction of the conditioned reflex when the morphine injections are discontinued takes place gradually, slower in some dogs, faster in others, the curve of extinction in the latter being a second degree parabola.

9. Reëstablishment of the conditioned reflex after it had been abolished is accomplished in less time than the establishment of the reflex, confirming Anrep.

10. The establishment, extinction and reëstablishment of the conditioned reflex resemble closely learning, forgetting and relearning Ebbinghaus nonsense syllables.

11. The conditioned reflex may be imperfectly established if the application of the combination of the conditioned and the unconditioned stimuli is alternated with the application of the conditioned stimulus alone.

12. Starvation acts deleteriously upon the course of the fully established conditioned reflex, or prevents its proper development, confirming Froloff.

13. Secretion of saliva is first stimulated, then depressed by the injection of morphine. Salivation generally stops in 15 to 20 minutes.

14. The greater the rate of the conditioned secretion of saliva the sooner will the depression produced by morphine set in.

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CALCIFICATION STUDIES WITH PIGS FED DIFFERENT PROTEIN SUPPLEMENTS

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In previous studies Maynard, Coldberg and Miller (1925a, 1925b) showed that a low-calcium, high-phosphorus, basal ration consisting of yellow corn, wheat middlings, linseed oil meal and common salt produced in growing pigs a bone very low in mineral constituents as compared to the bone developed when the basal ration was supplemented with bone meal and ground limestone to increase its calcium content. However, an even better bone was produced when menhaden fish meal, a feed rich in calcium and carrying considerable amounts of phosphorus also, was substituted on an equal protein basis for the linseed meal in the basal ration. The amounts of calcium and phosphorus and the ratio existing between these elements in the fish meal ration did not differ markedly from the amounts and ratio in the supplemented basal ration and the question therefore arose as what factor was responsible for the better calcification produced with the fish-meal ration.

From the make-up of the supplemented basal ration one would expect it to be low in the specific factor aiding calcium assimilation and some experimental evidence for this view was obtained in the previous studies (1925a). Thus, one possible explanation of the better calcification obtained when the fish meal was substituted in the basal ration was considered to be that this feed increased the amount of anti-rachitic factor in the ration.

In looking for a further explanation, the question as to the significance of the substitution of an animal-protein supplement for the linseed oil meal presented itself. In addition to a greater calcification the fish-meal ration produced somewhat more rapid gains in weight and a better condition in general than did the ration having similar mineral relations but containing linseed meal as the protein supplement. This is in accord with the generally observed fact that a ration containing an animal-protein supplement will produce more satisfactory growth in pigs than can be obtained with a ration of similar protein content, made up entirely from plant sources. From these considerations the question arose as to whether the better calcification obtained with the fish-meal ration was connected in any

significant way with the more rapid growth resulting from the use of the animal-protein supplement, and specifically, whether it was favorably influenced by the qualitatively better protein intake or by some other factor contributing to the better growth obtained. These considerations raised questions as to the inter-relations between such factors as rate of growth, quality of protein, calcium and phosphorus supply, and calcifica-

TABLE 1

Composition of rations

RATION NUMBER	INGREDIENTS	CALCIUM	PHOSPHORUS	NUTRITIVE RATIO
		per cent	*per cent*	
1	200 lbs. Yellow hominy 100 lbs. Wheat middlings 54 lbs. Fish meal 15 lbs. Corn starch 2 lbs. Salt	1.106	1.143	1:4.8
2	200 lbs. Yellow hominy 100 lbs. Wheat middlings 75 lbs. Linseed oil meal 5 lbs. Steamed bone meal 5 lbs. Ground limestone 2 lbs. Salt	0.868	0.937	1:4.8
3	200 lbs. Yellow hominy 100 lbs. Wheat middlings 10 lbs. Blood flour 20 lbs. Casein 40 lbs. Corn starch 2 lbs. Salt	0.190	0.743	1:4.8
4	200 lbs. Yellow hominy 100 lbs. Wheat middlings 10 lbs. Blood flour 20 lbs. Casein 40 lbs. Corn starch 5 lbs. Steamed bone meal 4 lbs. Ground limestone 2 lbs. Salt	0.916	0.967	1:4.8

tion, in the previous studies, and indicated the desirability of further and more specific studies along these lines.

In planning further work to test out the significance of the various explanations suggested as to the reason for the better calcification obtained with the fish-meal ration a need was felt for experiments more carefully controlled with respect to the make-up of the ration and to other factors

than was possible in the trials with pigs as previously conducted. Thus, it was planned that the further studies should be carried with rats as well as pigs, and related experiments with both species were therefore begun.

One series of studies with both species has been completed. The present paper describes the studies with pigs and in a following paper (Miller and Maynard, 1927) the experiments with rats are reported.

One object of the studies with pigs was to ascertain whether an animal-protein supplement of an entirely different nature from fish meal and one that was practically a pure source of protein would have the same influence as the fish meal in increasing the calcification when substituted for the vegetable-protein supplement in a ration having suitable calcium and phosphorus relations. Another object was to ascertain the extent of the calcification produced with a ration containing an animal-protein supplement with a low-calcium, high-phosphorus ratio, to compare the results obtained with those previously observed with a ration with a similar ratio but made up entirely from plant sources, and also to compare the results with those from the animal-protein ration with a corrected ratio.

EXPERIMENTAL PROCEDURE. The rations used and their analyses are shown in table 1.

The first two rations are similar to those used in the previous studies (1925a, 1925b) with the exception that yellow hominy feed is used in place of yellow corn meal. This change was made to make the ration more bulky and thus of a better physical character for young pigs. Hominy feed is very similar to corn meal in analysis and in feeding value for pigs and thus the change was not considered to alter the essential character of the rations to any marked degree.

Rations 1 and 2 were formulated to be similar with respect to their content of calcium and phosphorus and to supply these elements in adequate amounts and in approximately a 1:1 ratio.

For comparison with rations 1 and 2 it was desired to have a ration containing an animal-protein supplement of an entirely different character from the fish meal, as it has been stated. It was also desired that this supplement should be of such a calcium and phosphorus content that one ration could be made in which the ratio between these two minerals would be similar to that existing in the previously used vegetable-protein ration containing no mineral additions, and that another ration of a mineral content similar to rations 1 and 2 could be made, by an appropriate addition of the mineral supplements. Further it was desired that this protein supplement should be as pure a source of protein as would meet the other specifications yet be obtainable at a price making its use possible in the amounts needed. To meet these various requirements a mixture of two parts of casein and one part of blood flour was selected. Neither of these materials alone would meet the specifications as to calcium and phosphorus

content and the proportion in which they were combined was chosen to provide the desired mineral relations. On the basis of its nitrogen content, this mixture was calculated to contain approximately 95 per cent of protein on a moisture-free basis.

Substituting this animal-protein mixture on an equal protein basis for the fish meal in ration 1 and increasing the starch component to keep the nutritive ratio the same, provided a ration, number 3, having a calcium-phosphorus ratio of 1:4, a ratio similar to that in the vegetable-protein basal ration which produced the poorest calcification in previous experiments. By an appropriate addition of bone meal[1] and limestone to ration 3, ration 4 was formulated, having calcium and phosphorus similar in amount and in ratio to ration 1.

The various rations shown in table 1 are seen to be similar as regards nutritive ratio, and this ratio is a proper one for the growth of young pigs, according to accepted feeding standards. The amounts of digestible protein furnished by the protein supplements are similar in all cases. The rations are adequate as regards vitamins A and B. Vitamin C is not needed for pigs.

Rations 1 and 4 are similar in having animal-protein supplements, and in their calcium and phosphorus content, but differ from ration 2 in that the latter has a vegetable-protein supplement. Thus comparisons between rations with these two kinds of supplements but with similar mineral relations are provided.

Rations 3 and 4 provide for a comparison between rations alike as regards their animal-protein supplements, but with widely different calcium-phosphorus ratios.

The rations also provide for comparisons with the results of the previous experiments.

Twenty purebred Duroc-Jersey pigs, 5 sows and 15 barrows, farrowed in the University herd were used in this experiment. Thrifty pigs, weighing 25 to 40 pounds at 60 to 75 days of age, were selected and separated into groups of five, to provide one group for each ration shown in table 1. As many litter mates as possible were used and were distributed among the groups to be compared.[2] In tables 2 and 3 the pig numbers that have the

[1] A special product manufactured by the United Chemical and Organic Products Co., Chicago, Ill.

[2] While the question of litter mates may not be an important one in selecting experimental animals from a colony of white rats subjected to the same system of feeding and breeding for several generations, it is a consideration worthy of attention with a herd of swine. With the latter, breeding and selection are constantly being practiced and considerable difference in type exist among the animals of a given breed. These differences show in variations in ability to grow, in conformation and in quality of bone. Thus, distribution of litter mates among the various rations tends to lessen the experimental error due to individual variation.

same letter prefixed represent litter mates. One pig allotted to ration 3 proved unthrifty and was removed from the experiment on the 40th day, thus tables 2 and 3 show only 4 pigs for this ration.

From weaning time until placed on experiment the pigs had been fed alike with liberal amounts of skim-milk and grain. They had been exposed to direct sunlight at various times during the three weeks prior to the start of the experiment. On the basis of their previous feeding and management,

TABLE 2

Average daily gains in weight

RATION NUMBER	PIG NUMBER	AVERAGE DAILY GAINS DURING 100 DAYS
		pounds
1	A 935	1.09
	A 937	0.90
	B 977	1.14
	C 961	1.13
	D 953	1.16
	Group average	1.08±0.03
2	A 933	1.04
	B 974	0.71
	B 979	0.76
	C 960	1.16
	E 922	0.88
	Group average	0.91±0.06
3	A 936	0.98
	B 978	1.17
	C 962	0.75
	F 918	1.03
	Group average	0.98±0.06
4	A 931	1.07
	A 934	1.19
	B 972	1.20
	C 963	1.14
	D 950	1.02
	Group average	1.12±0.03

as well as from their size and condition, they were considered in an excellent state of nutrition when placed on experiment.

At the beginning of the trial all of the pigs were given two treatments of oil of chenopodium with castor oil for intestinal parasites. This proved to be an effective treatment inasmuch as upon autopsy at the end of the experiment Ascaris Lumbricoides were found in only one pig.

The pigs were housed in four adjoining pens on the north side of a colony

house and were never exposed to direct sunlight after the start of the experiment. They were fed all that they would eat three times a day. At ten-day intervals they were weighed and regular observations were made as to their appearance and general condition.

It was planned to continue the feeding trial for approximately four months as was done in the previous studies and then to slaughter the pigs,

TABLE 3

Ash content of femurs

RATION NUMBER	PIG NUMBER	AGE AT END OF EXPERIMENT	PERIOD ON EXPERIMENT	LIVE WEIGHT	WEIGHT OF FRESH FEMUR	COMPOSITION OF FRESH FEMUR	
						Dry matter	Ash
		days	*days*	*lbs.*	*grams*	*per cent*	*per cent*
1	A 937	190	117	148	203.9	62.87	24.87
	A 935	183	110	157	211.3	69.48	25.21
	B 977	185	125	180	228.4	64.67	26.90
	C 961	179	100	145	207.0	66.62	27.17
	D 953	193	125	166	254.2	65.30	27.18
					Group average		26.27±0.41
2	A 933	190	117	167	245.6	60.54	19.66
	B 974	192	131	137	183.4	67.39	24.42
	B 979	185	125	120	182.0	61.26	21.40
	C 960	179	100	148	194.9	57.36	19.59
	E 922	199	138	175	222.7	63.76	24.96
					Group average		22.01±0.91
3	A 936	183	110	143	177.5	55.33	15.89
	B 978	192	131	182	207.0	57.78	18.33
	C 962	179	100	110	158.7	47.45	17.31
	F 918	201	138	154	199.0	59.15	16.39
					Group average		16.98±0.41
4	A 931	190	117	170	243.5	63.33	22.76
	A 934	183	110	153	212.0	66.37	25.35
	B 972	192	131	187	232.6	64.19	27.45
	C 963	179	100	154	220.4	66.47	25.35
	D 950	207	138	180	248.7	67.43	27.53
					Group average		25.69±0.61

and following a routine post-mortem examination, to save the femurs for chemical analysis.

In accordance with this plan the pigs were placed on the experimental rations November 6, 1925, and slaughter was begun 100 days later on February 13. Facilities were not available for slaughtering all of the animals at the same time. On February 13 a litter mate from each ration was killed, choosing the oldest group of litter mates. Each succeeding week

a further selection from each ration was made for slaughter, taking litter mates in so far as available and reserving the youngest pigs until the last. Thus 5 weeks elapsed from the time the first pigs were killed until the final

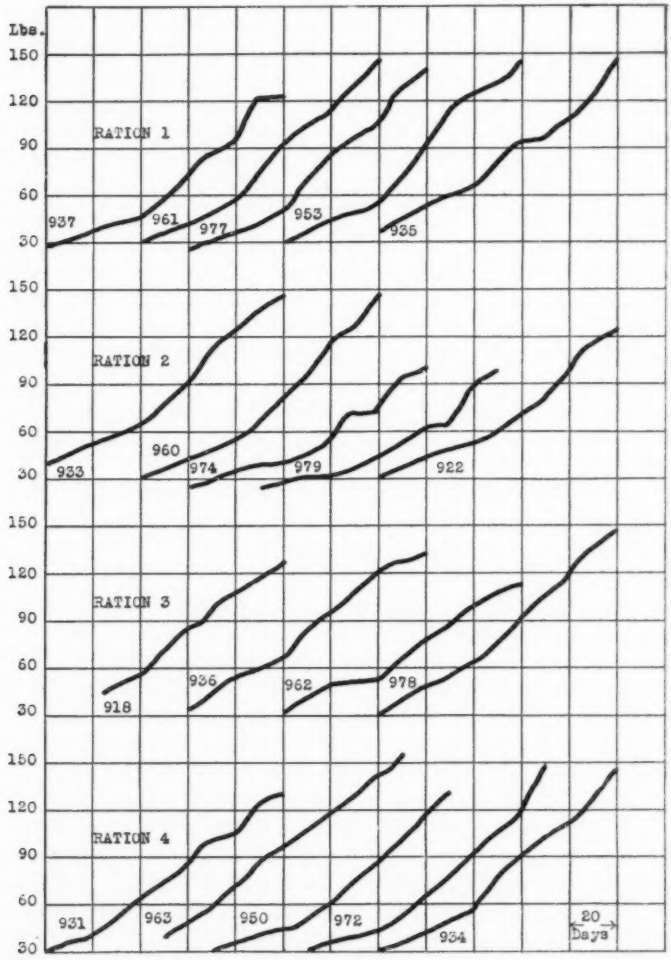

Fig. 1. Curves of growth in weight

group of four was disposed of. This accounts for the differences in days on experiment as shown in table 3.

For chemical analysis the femur was freed from adhering flesh, partially

dried at 50° to 60°C., and then dried to constant weight over sulfuric acid. The bone was next pulverized in a bone cutter, sampled, and the sample ignited at dull redness in an electric muffle furnace to determine the ash.

The femurs were analyzed for dry matter and total ash. It was not deemed necessary to determine the calcium and phosphorus in the ash because in one of the previous studies (1925a) it was shown that these two elements remain practically constant in ratio, and that changes in total ash content represent corresponding changes in calcium and phosphorus.

RESULTS. The growth curves are shown in figure 1. It is seen that all of the pigs grew fairly regularly but that those on the rations that contained both an animal-protein supplement and a proper calcium-phosphorus ratio, namely, rations 1 and 4, excelled somewhat both in rate and regularity of growth. This is further brought out in table 2 where the average daily gains are given. Attention should be called to the fact that the data in figure 1 and in table 2 are for 100 days only in the case of all the animals, although most of them were on experiment one to four weeks longer. Since slaughter was begun at the end of the 100-day period it represents the longest time that each group as a whole was on experiment, and it seemed desirable to choose this uniform period in order to average the individual gains.

The animals on ration 1 showed the best physical development and condition of any of the groups, as indicated by sleek, shiny coats, the desired length of body, and excellent appearance in general. Those on ration 4 ranked next in these respects.

The data in table 2 show that the group of pigs on ration 2, containing the vegetable-protein supplement, failed to make as good growth in weight as those on the rations containing animal-protein supplements. This is in accord with previous results. These pigs had rather rough coats and did not show as good body development as those on rations 1 and 4.

The data for ration 3 show that a very deficient supply of calcium had little influence on growth in weight since the group on this ration showed nearly as large an average daily gain as the pigs on ration 4 and a larger daily gain than those on ration 2. Though ration 3 was adequate for the growth of flesh, its inadequacy for the growth of bone was clearly evident in the general condition and body development of the pigs. Though the pigs on this ration grew rapidly from the start they were early noted to be developing shorter bodies than normal and they presented a dumpy appearance. On the 90th day of the experiment the characteristic stiffness, which in the previous experiments was found to be correlated with poor bone growth, made its appearance with three of the animals in this group. The fourth member of the group developed this same stiffness a little later. In no case was the stiffness as severe as was frequently noted in the earlier

work with a ration low in calcium and containing a vegetable-protein supplement, and none of the pigs lost the ability to rise or to stand on their feet. No stiffness developed with any of the other rations in this experiment.

It should be pointed out in connection with the foregoing discussion of the data in table 2 that one is not justified in drawing general conclusions as to rates of growth of pigs where so few animals are involved. The principal object in presenting these data is to consider them in interpreting the results of the calcification.

Sherman and MacLeod (1925) have shown that when rate of growth is decreased due to a shortage of cystine the calcification of the bones is not retarded as greatly as is increase in weight and consequently on a percentage basis the body of the slower growing rat is higher in calcium. It is therefore clear that in studies of the influence of various rations on calcification in growing animals as carried out in the experiments here reported the rate of growth must be considered in interpreting the results. The results will have the most exact significance when the animals being compared grow rapidly and at a similar rate while widely different rates of growth will introduce a variable tending to prevent the drawing of definite conclusions from the calcification data.

From this point of view the most important fact brought out in the data in figure 1 and in table 2 is that all of the animals made fairly satisfactory gains, at least tripling their weights, and that they were growing regularly at the time they were killed for a study of the bones. Thus the factor of variation in growth does not enter into the results of the calcification studies to any great degree. The possible significance of the actual differences in rate of growth of the different groups as shown in table 2 will be discussed later.

In table 3 the results of the bone analyses are shown. The third column of the table shows that the pigs were approximately the same age when killed, an important consideration since bone composition normally varies with age. The next column shows the extent of the variable factor resulting from not killing all of the animals on the same day. In this connection, however, it should be remembered that, due to the method of selecting the animals for each killing, the average number of days on experiment was the same for each group, and that comparison can be made between individuals, usually mates, killed on the same day.

Turning to the figures for percentage of ash it is seen that the highest percentages were produced with rations 1 and 4 and that these two rations are practically identical in this respect. The ash content of the femurs from the pigs receiving a ration with the same favorable calcium-phosphorus ratio but having a vegetable-protein supplement (ration 2) is distinctly lower. This is seen to be true in the case of each lot of litter

mates compared and the probable errors noted between group 2, and groups 1 and 4 are significant. The results here obtained are in entire agreement with those of the previous experiments where rations similar to 1 and 2 were used.

The results with ration 3 are most striking. Here animals which grew nearly as well as those on ration 4, and received identical food with the exception of the calcium factor and the resultant change in the calcium-phosphorus ratio, developed a bone containing approximately 35 per cent less ash. The findings with respect to this ration are in accord with what was expected from the external symptoms that developed in the pigs receiving it.

The large increase in ash content of the femurs produced by ration 4 compared to that produced by ration 3 and brought about by the addition of calcium is in entire agreement with the increase that resulted in the previous trials (1925a, 1925b) in which rations containing a vegetable-protein supplement were fed, with and without appropriate calcium content. The two series of results check each other in showing the striking increase in calcification in growing pigs which is brought about by an appropriate adjustment of the calcium and phosphorus content, even when the ration is presumably very low in vitamin D.

Although the mineral relations of the food would appear to be the primary factor governing the extent of calcification of the bones of growing pigs, the consistently higher figures for ash content obtained with rations 1 and 4 than with ration 2 indicate that the substitution of an animal-protein for a vegetable-protein supplement in some way favorably influenced calcification. This is in accord with the previous results with the fish meal ration. Further evidence for this conclusion is furnished by the data obtained with ration 3. The average per cent of ash for the group on ration 3, 16.98 per cent, is higher than the figure, 14.41 per cent, representing the average of 8 values obtained in the previous studies (1925a, 1925b) using a ration having a similar calcium-phosphorus ratio but containing linseed oil meal instead of an animal-protein supplement. Taken separately this comparison is of limited significance since the two rations were fed in different years and differed somewhat in make-up, but considered with the other comparisons previously mentioned confirmatory evidence is furnished thereby.

That the better calcification obtained with the rations containing animal-protein supplements was due to the nature of the protein per se or to the somewhat better growth in weight occurring in these groups does not seem a reasonable explanation in view of the results of Sherman and MacLeod (1925). These investigators found that where growth was retarded due to a protein intake deficient in quality the body contained more calcium on a percentage basis than was the case with a more rapidly growing animal.

On this basis it would be expected that the bones of the pigs receiving the animal-protein supplements would show a lower percentage of ash than those of the groups receiving the vegetable-protein ration. The actual results were just the opposite of this.

That the better calcification obtained with the fish-meal ration was due to vitamin D supplied by the fish meal is an explanation which is suggested by the studies with rats (Miller and Maynard, 1927) which were carried out in conjunction with the studies here reported. No study has been made to ascertain whether the same explanation might apply in the case of the ration supplemented with casein and blood meal. Additional experiments to study this and other questions raised by the results thus far obtained are contemplated.

SUMMARY

In studies with growing swine, using the ash content of the femurs as the measure of calcification, it was found that better calcification was caused by a ration containing fish meal as the protein supplement and also by a ration containing blood meal and casein as the supplements than by a ration containing linseed oil meal as the protein supplement. All of these rations were similar as regards their content of calcium and phosphorus which were supplied in approximately a 1:1 ratio, ground limestone and bone meal being used to help provide these minerals in the oil-meal ration and in the casein-blood-meal ration. All of the rations caused rapid and nearly equal growth, but the oil-meal ration was slightly surpassed by the other two in this respect.

With a fourth ration, identical with the casein-blood-meal ration with the exception that its calcium content was greatly lowered and its phosphorus content slightly by the omission of the mineral supplements, the rate of growth was nearly equal to that caused by the same ration with the mineral supplements, but the ash content of the femurs was approximately 35 per cent less and the characteristic external symptoms of inadequate mineral nutrition developed.

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CALCIFICATION STUDIES WITH RATS FED MENHADEN OIL AND VARIOUS MENHADEN FISH MEALS

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In another paper the writers (1927) have reviewed some of their studies with growing pigs showing that with rations approximately alike as regards calcium and phosphorus content a greater calcification occurred when menhaden fish meal was the protein supplement than when linseed oil meal was the supplement used, and in this same paper further studies with pigs are reported. The experiments described in the present paper were carried on contemporaneously with those reported in the paper referred to above and were undertaken to ascertain whether menhaden fish meal contains the specific factor aiding calcium assimilation.

Menhaden fish meal is obtained from the menhaden fish, Brevoortia Tyrannus. The whole fish are usually cooked to break down the moisture and oil cells, some menhaden oil being obtained thereby. Further extraction of the oil is accomplished by pressing, the residue is then dried and ground, the product being known as fish meal.

In an earlier report from this laboratory (Miller, Wohlwend and Maynard, 1926) some preliminary observations on the influence of menhaden fish meal and menhaden oil on bone calcification in rats were described. The rachitic ration described by Steenbock and Black (1925) consisting of yellow corn 76, wheat gluten 20, calcium carbonate 3 and sodium chloride 1, was used as the basal ration. The influence of additions of the fish meal and the oil on the calcification produced by this ration was determined by comparative studies of the ash content of the leg bones after various periods of growth. The addition of 2 mgm. daily of menhaden oil did not result in any increase in ash content over that produced by the basal ration. However a group of rats fed the basal ration plus 16.6 per cent of fish meal showed an average ash content of 57.14 per cent in the leg bones, on a moisture-free, fat-free basis, as compared to an average value of 40.19 per cent for the group on the basal ration. However the addition of the fish meal increased the calcium and phosphorus content of the basal ration and also changed the phosphorus-calcium ratio from 1:4.5 to 1:2.5. In view of the more favorable calcium and phosphorus relations thus established the writers did not feel justified in concluding

that the better calcification produced on the addition of the fish meal was due to the presence in the fish meal of the specific factor aiding calcium assimilation. Thus further studies were undertaken and are here reported.

Experiments with menhaden fish meal. The rations used in the present studies with fish meal are shown in table 1.

Ration 1 is the ration of Steenbock and Black (1925) used as the basal one in the previous studies. Ration 17 is the same ration so modified as to calcium and phosphorus content as to permit the substitution of fish meal in it without changing the percentages of these minerals, with the object of eliminating this varying factor which prevented the drawing of definite conclusions from the previous results. In order to provide for the desired uniform calcium and phosphorus content it was necessary so to

TABLE 1

Compositions of rations

	RATION NUMBER					
	(1)	(17)	(18)	(19)	(20)	(21)
Yellow corn	76	73.5	73.5	73.5	73.5	73.5
Wheat gluten	20	20.0	7.0	7.0	7.0	7.0
Fish meal			15.0	15.0	15.0	15.0
NaCl	1	1.0	1.0	1.0	1.0	1.0
$CaCO_3$	3	0.3	3.0	3.0	2.5	3.0
$Ca_3(PO_4)_2$		2.5	0.5		0.5	
Calcium per cent	1.228	2.195	2.178	2.169	2.178	2.136
Phosphorus per cent	0.272	0.772	0.768	0.744	0.764	0.771
P:Ca Ratio	1:4.51	1:2.84	1:2.84	1:2.91	1:2.85	1:2.77

increase the content of these minerals in the basal ration that upon the inclusion of the fish meal they could be withdrawn in amounts sufficient to offset the additions contained in the amount of fish meal added. Rations 18, 19, 20, and 21 each contain 15 per cent of a different brand of menhaden fish meal.[1]

The data in table 1 show that these rations contain the same percentages of calcium and phosphorus as ration 17. In adding the fish meal, sufficient of the wheat gluten was withdrawn from ration 17 to keep the protein content of the various rations the same.

The measure of calcification used was the ash content of the leg bones. For the ash determinations the femur and tibia were excised, freed from

[1] The various fish meals used in this experiment were supplied to us by the C. L. Struven Co. of Baltimore, Md., the Trition Oil and Fertilizer Co., of New York, the Park and Pollard Co., of Buffalo, N. Y., and the Royster Guano Co., of Baltimore, Md.

adhering tissue and combined into a single sample which was dried, crushed, and extracted with alcohol. The extracted bones were then freed from moisture by drying at 100°C., and ashed in a muffle furnace at dull red heat. The results of the ash determination as given in this paper are therefore on an extracted, moisture-free basis.

Six groups of 5 rats each, weighing 38 to 45 grams at 22 to 26 days of age were used in this study. The six groups of rats were fed for a period of five weeks, at the end of which ash determinations were made. In addition, 5 rats from the same litters from which the animals were selected were killed at the beginning of the experiment for the determination of ash in the leg bones. This was done to secure a figure that could be considered to represent the ash content of the bones of the experimental animals when placed on experiment. The growth curves are shown in figure 1. The results of the ash determinations are given in table 2.

In this table the group numbers correspond to the ration numbers in table 1. The ash content of the bones of the rats killed at the beginning of the experiment is higher than has been reported by other investigators. However, this figure has been confirmed in this laboratory and it is believed to represent a true picture as regards the experimental animals when placed on experiment. The diet of the stock rats in this colony has remained unchanged for over four years.[2]

It is seen in table 2 that over the five-weeks' period the ash content of the leg bones of the rats in group 1 fed the unmodified basal ration markedly decreased compared to the figure for the check group killed at the start. When the modified basal ration (group 17) was fed an increase in ash occurred compared to the figure representing conditions at the start, and a much larger and remarkably uniform calcification is shown for the four groups receiving the rations containing fish meal. Comparing the results with groups 1 and 17 it is evident that a phosphorus-calcium ratio of 1:2.84 favors calcification to a greater extent than does a ratio of 1:4.51, the rations being the same in other respects. The growth curves (fig. 1) of these two groups of rats are very similar.

Comparing the results with groups 18 to 21 inclusive, with those for group 17 where the phosphorus-calcium ratio remained unchanged, it is

[2] Except for periodic additions of cod liver oil, the ration fed has been as follows:

Yellow corn meal	25
Wheat red dog flour	22
Oat flour	15
Linseed oil meal	15
Ground malted barley	10
Soluble blood flour	10
Ground limestome	1
Steam bone meal	1
Sodium chloride	1

evident that the fish meal supplied a factor that increased calcification. This finding furnishes an explanation of the favorable effect of a fish-meal ration on calcification in growing pigs as described in a previous paper (Maynard and Miller, 1927).

The rations including fish meal caused more rapid growth than the basal rations. This fact is considered by the writers to be of importance in interpreting the results. Sherman and MacLeod (1925) have shown that when growth is retarded, calcification is retarded to a lesser degree and that on a percentage basis the body of the slower growing rat is higher in

TABLE 2

Percentage of ash in leg bones

GROUP NUMBER	NUMBER OF RATS PER GROUP	ASH IN LEG BONES (DRY, FAT-FREE BASIS)
		per cent
Check group killed at beginning of experiment	5	48.18±0.77
1	5	43.39±1.35
17	5	53.80±0.82
18	5	59.21±0.27
19	5	58.62±0.39
20	5	59.14±0.51
21	5	59.42±0.41

TABLE 3

Percentage of ash in leg bones

GROUP NUMBER	NUMBER OF RATS PER GROUP	ASH IN LEG BONES (DRY, FAT-FREE BASIS)
		per cent
Check group killed at beginning of experiment	5	49.16±0.71
Basal	5	43.39±1.35
Basal + 10 mgm. menhaden oil daily	5	52.96±0.89
Basal + alcoholic extract of fish meal	5	53.94±0.53

calcium. On this basis with the other conditions influencing calcification being equal one would expect the bones of the rats in group 17 to be higher in ash than those in groups 18 to 21. However, despite their much faster growth, the rats in the latter groups showed a markedly greater calcification and the conclusion that the fish meal supplied a factor favoring calcification is strengthened by this consideration.

Experiments with menhaden oil. There remains in menhaden fish meal 4 to 8 per cent of oil. The question therefore arose as to whether the factor aiding calcification was present in the oil fraction. To study this

question experiments were carried out with menhaden oil furnished by the C. L. Struven Co., Baltimore, Maryland, a product expressed from the same batch of fish from which the meal used in ration 17 was obtained. It was felt that if the oil itself was found to promote calcification this would constitute further evidence that the fish meal containing this oil also possessed this property. It was possible to plan experiments with the oil using the rachitic ration of Steenbock and Black without encountering the difficulties met with the fish meal due to its content of calcium and phosphorus. It was recognized, however, that changes modifying the properties of the oil might result in the drying of the fish meal after the oil is expressed. Therefore it was planned to include an alcoholic extract of the fish meal in the study to ascertain whether the oil remaining in the meal had a similar effect to that which had been expressed.

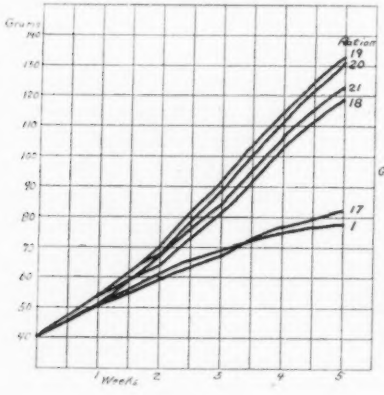

Fig. 1

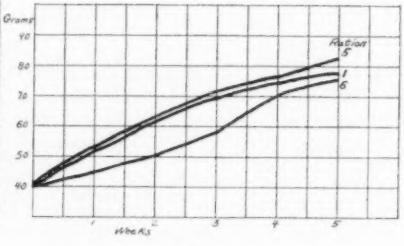

Fig. 2

Fig. 1. Growth curves. Fish-meal experiments
Fig. 2. Growth curves. Menhaden oil experiments

The ration of Steenbock and Black, listed as ration 1 in table 1, was used as a basal ration. A group of 5 rats received in addition to the basal ration 10 mgm. daily of menhaden oil, and another group received daily, in addition to the basal ration, an amount of an alcoholic extract of the fish meal which was approximately equivalent to 10 mgm. of menhaden oil. This extract was prepared daily and incorporated in the basal ration in amounts which were consumed daily. The ration was thoroughly air-dried before being fed.

The rations were fed for a period of five weeks, at the end of which the ash content of the leg bones was determined as in the studies with fish meal. The growth curves of the various groups are given in figure 2 and the results of the ash determinations are given in table 3.

The figure shown for the ash content of the bones of the check group killed at the start of the experiment is in close agreement with the similar figure in table 2.

The figure for ash content of the leg bones of the rats on the basal ration is the same one reported in table 2. It did not seem necessary to repeat the determinations for this ration since the figure reported in table 2 was obtained under identical experimental conditions and since the reliability of this figure was further supported by the fact that similar figures were obtained in the preliminary experiments (Maynard and Miller, 1927) under identical conditions. The growth curve for ration 1 shown in figure 2 is also based on data obtained in the experiments with the fish meal.

From the data in table 3 it can be seen that the percentage of ash in the leg bones of the rats receiving the basal ration decreased greatly during the five-weeks' period while those receiving the menhaden oil and the alcoholic extract of fish meal increased to a certain extent. Thus it is indicated that both of these substances supplied the factor aiding calcification. In the preliminary experiments (Maynard and Miller, 1927) the addition of 2 mgm. of menhaden oil daily to the basal ration resulted in no better calcification than the basal ration alone, although a similar addition of cod liver oil resulted in some improvement in this respect. One would not expect a body oil to be as rich as a liver oil in the antirachitic factor, but that menhaden oil does contain this factor to a certain extent seems clear from the results of the present experiment where the much larger daily addendum was used. It also appears that this factor that is in the oil remaining in the residue after pressing is not destroyed by the further processing of the residue. Thus these results substantiate those with the fish meals as reported earlier in this paper.

The composite growth curves in figure 2 show that the rats on the ration with the addition of the alcoholic extract did not grow as rapidly during the first part of the experimental period as did the other groups. While records of food intakes were not kept it was the writers' observation that this ration proved rather unpalatable and was not consumed in as large amounts as the others at the start. Later an increased consumption was apparent and the growth curve shows that the early poor growth was largely offset by a more rapid growth later. Since at the time the rats were killed for analysis of the bones the average weight of the animals on ration 6 was nearly identical with the figure for the group on the basal ration it is not believed that the earlier differences in growth constitute a disturbing factor in the interpretation that has been placed on the data in table 3.

The rates of growth shown in figure 2 are very much smaller than those in figure 1 for the groups receiving fish meal in their rations. Although the rations containing fish meal had a more favorable calcium-phosphorus

ratio than those containing the menhaden oil or the alcoholic extract, this more favorable ratio does not appear to be the explanation of the better growth with the fish-meal rations, because ration 17 having the same ratio as the latter rations, but containing no fish meal, resulted in no better growth than rations 5 and 6 having the much wider calcium-phosphorus ratio. Evidently the better growth obtained with the fish-meal rations was due to the higher quality of the protein in these rations even as was found to be true in the pig studies (Maynard and Miller, 1927). It was not due to any specific factor present in menhaden oil or extractible from fish meal by alcohol, in view of the slow growth on rations 5 and 6 as compared to that with rations 18 to 21.

SUMMARY

Studies are reported with growing rats using the ash content of the leg bones as the measure of calcification, from which it is concluded that menhaden fish meal and menhaden oil contain the specific factor aiding calcification.

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THE EFFECT OF FORCING FLUIDS UPON SURVIVAL AFTER BILATERAL EPINEPHRECTOMY

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Previous work by several investigators has shown that the administration of certain solutions may result in prolongation of life beyond the usual period in epinephrectomized animals. Thus, Stewart and Rogoff (1925), working on dogs, report that intravenous injections of Ringer's solution and dextrose prolong life. Zwemer (1925), working on cats in this laboratory, was able to keep his double-operated animals alive for considerable periods by administering a 5 per cent solution of glucose orally. This work suggested the possibility that forcing fluids containing various substances, or even water alone, might prove beneficial in the treatment of adrenal insufficiency following gland removal. Both Zwemer (1926) and Swingle (1926), in this laboratory, have noted and commented upon the symptoms of dehydration which appear after bilateral epinephrectomy in cats, and the fact that animals presenting marked signs of adrenal insufficiency eagerly drink large quantities of water up to within a few hours of death. Their observations lent further support to the idea that forcing fluids might prove efficacious.

The writer takes this opportunity of expressing his obligation to Prof. W. W. Swingle for suggesting the problem and for kindly criticism during the progress of the work.

The oral method for administration of fluids was adopted. By means of the stomach tube, solutions were given to the animal beginning immediately after the second operation, and the treatment was continued until the appearance of symptoms immediately premonitory to death. The passage of the tube when an animal was in, or verging on, coma, was found to be useless, as the excitation almost invariably led to convulsions and death within a few minutes. In order to pass the stomach tube—a rubber catheter of suitable diameter—the animals were placed in a wooden box so constructed that the head alone protruded. The box was of such size as to allow the animals a minimum of movement coincident with comfort. A rubber gag, composed of a short piece of tubing threaded on a stout cord, was next inserted into the mouth of the animal and the ends of the cord tied behind the ears. This prevented puncturing of the tube due to

TABLE 1

NUMBER	SEX	DAYS ELAPSED BETWEEN FIRST AND SECOND OPERATION	SURVIVAL AFTER SECOND OPERATION	COMPLICATIONS
		Section I		
Ringer's solution. Treatment: 50 cc. Ringer's solution twice daily, 50 cc. milk once daily.				
			hours	
1	Male	9	216.0	None
2	Female	8	132.0	None
3	Female	7	62.0	Abnormal, large, cyst at site of kidney, complicating operation
		Section II		
Ringer's solution (each constituent double strength). Treatment: 50 cc. Ringer's solution twice daily, 50 cc. milk once a day.				
4	Male	7	188.0	None
5	Female	7	96.0	None
		Section III		
Solution "1": Magnesium lactate, 0.9 per cent; strontium chloride, 0.024 per cent; sodium (acid) phosphate, 0.042 per cent. Treatment: 50 cc. solution "1" twice daily, 50 cc. milk once daily.				
6	Female	9	341.0	Stitch infection
7	Male	7	107.0	None
8	Male	7	196.0	None
9	Female	7	125.0	None
10	Female	3	91.5	Wound infection
		Section IV		
Solution "1-A": Magnesium lactate, 0.9 per cent; strontium lactate, 0.024 per cent; sodium (acid) phosphate, 0.042 per cent. Treatment: 50 cc. solution "1-A" three times daily, 50 cc. milk once daily.				
11	Female	7	162.5	None
12	Male	7	137.5	Wound infection
		Section V		
Solution "1-B": Magnesium sulphate, 0.9 per cent· strontium chloride, 0.024 per cent· sodium sulphate, 0.042 per cent. Treatment: 50 cc. solution "1-B" twice daily, 50 cc. milk once daily.				
13	Female	7	18.0	Broke ligature-hemorrhage
14	Male	5	68.0	None
15	Male	5	69.5	None

TABLE 1—*Continued*

AVERAGE PROLONGATION	WEIGHT AFTER SECOND OPERATION	WEIGHT AT DEATH	TOTAL LOSS
		Section I	
	grams	*grams*	*grams*
174 hours excluding no. 3	Not recorded	Not recorded	Not recorded
	Not recorded	Not recorded	Not recorded
	Not recorded	Not recorded	Not recorded
		Section II	
	grams	*grams*	*grams*
142 hours	4350	4020	330
	Not recorded	Not recorded	Not recorded
		Section III	
192.25 hours, excluding no. 10	Not recorded	Not recorded	Not recorded
	Not recorded	Not recorded	Not recorded
	3225	3025	200
	1900	1850	50
	2250	2200	50
		Section IV	
150 hours	2260	2220	40
	2428	2190	238
		Section V	
68.75 hours, excluding no. 13	Not recorded	Not recorded	Not recorded
	2953	2645	308
	2983	2690	293

TABLE 1—*Continued*

NUMBER	SEX	DAYS ELAPSED BETWEEN FIRST AND SECOND OPERATION	SURVIVAL AFTER SECOND OPERATION	COMPLICATIONS
				Section VI
Solution "1-C": Magnesium sulphate, 0.9 per cent; strontium chloride, 0.024 per cent; sodium (acid) phosphate, 0.042 per cent; ammonium chloride, 0.05 per cent; sodium chloride, 0.042 per cent; sodium bicarbonate, 0.042 per cent; glucose, 0.9 per cent. Treatment: 30 cc. solution "1-C" twice daily, 50 cc. milk once daily.				
			hours	
16	Male	5	177.0	None
17	Male	8	144.0	None
18	Female	9	47.0	Accidentally killed
				Section VII
Sodium chloride, 0.9 per cent. Treatment: 30 cc. sodium chloride three times daily, 50 cc. milk once daily.				
19	Male	13	168.0	None
20	Male	4	91.5	None
21	Female	7	187.5	None
				Section VIII
Sodium bicarbonate, 0.5 per cent. Treatment: 30 cc. sodium bicarbonate twice daily, 50 cc. milk once daily.				
22	Male	8	178.5	None
23	Female	8	163.0	None
24	Female	4	168.0	None
25	Male	7	80.0	Intestinal disorders—extreme diarrhea
				Section IX
Sodium acetate, 3.5 per cent. Treatment: 50 cc. sodium acetate three times daily, 50 cc. milk once daily.				
26	Female	7	42.0	Vomited everything given
27	Male	12	65.5	Stitch infection, vomited

chewing or biting. The stomach tube could then be passed without difficulty. Following a few passages of the tube, the animals took it quite readily with very little struggling. Without such an apparatus it was found to be practically impossible to administer solutions to a cat by stomach tube, and this arrangement is of particular value as the animals struggle very little—struggling and exertion always tending to aggravate the symptoms due to the extirpation of the glands.

Upon death the animals were autopsied for the presence of accessory

TABLE 1—*Concluded*

AVERAGE PROLONGATION	WEIGHT AFTER SECOND OPERATION	WEIGHT AT DEATH	TOTAL LOSS
		Section VI	
106.5 hours, excluding no. 18	2335	2310	25
	3565	3310	255
	2215	2202	13
		Section VII	
152.2 hours	3584	3192	392
	2770	2650	120
	2240	1950	290
		Section VIII	
147.3 hours	2870	2750	120
	1835	1740	115
	3270	3160	110
	2785	2655	130
		Section IX	
53.7 hours	2760	2600	160
	Not recorded	Not recorded	Not recorded

glands or any portion of glandular tissue not removed upon operation. Data upon such an animal showing either an accessory cortical gland, or bit of gland left behind at operation, were discarded as valueless in this study. In calculating weight losses, the weights were taken immediately after the second operation and at death, the difference in weight being recorded in the tables as the total weight loss.

Five to ten days was allowed to elapse between first and second operations. Zwemer (1926) and Swingle (1926) both observed that male cats

which have had both adrenals removed in two stages, 6 to 10 days apart, seldom survive over 60 hours after the second operation. Their data were obtained from animals operated upon during the fall and winter months. The present experiments were done simultaneously with those recorded in their papers and their data for untreated animals serve as checks for the treated animals reported upon here.

Previous investigation by Elliott and Tuckett (1906), Marshal and Davis (1916), and Zwemer (1925) had shown the necessity for adopting a definite time interval between the first and second operation when working upon the prolongation of life in bilaterally epinephrectomized animals. The investigators mentioned showed that if a considerable interval of time is allowed to elapse between the first and second operations the life-span of cats is considerably prolonged. Just why long intervals between operations should enable an animal to survive a greater length of time is unknown. It seems probable that minute bits of accessory cortical tissue may thus have time to hypertrophy and function to some degree, although at present there are no available data to substantiate this conclusion. It has long been known that when both adrenals are removed at one sitting death quickly results.

Various solutions were employed. The nature of the solution and data regarding each experiment are recorded in the tables. The food of the double operated animals was milk and this was given by stomach tube. Cats suffering from adrenal insufficiency rarely eat voluntarily after the first thirty hours following operation, and seem to have an especial loathing for meat.

None of the female cats employed in these experiments were pregnant. This is a point which must always be taken into consideration when dealing with any problem concerning survival periods of bilaterally epinephrectomized animals. H. A. Stewart (1913) was the first to clearly demonstrate that pregnancy definitely prolongs the life of the operated cats. More recently Stewart and Rogoff (1925) have confirmed this result in dogs.

In section I of the table, data are presented showing that the oral administration of Ringer's solution prolongs life considerably. The average life span of double operated animals—with an interval of seven days between operations, is about 60 hours. This figure is based on the data obtained from the records of one hundred animals operated upon in this laboratory by Swingle (1926) and Zwemer (1925–26). The animals were operated upon during the fall and winter months. If we exclude animal 3, obviously an abnormal case owing to the presence of an enormous cyst on the kidney site, the survival period averaged 174 hours. There can be little question but that Ringer's solution aids in prolonging life in epinephrectomized cats.

It is interesting to note that doubling the percentage of the constituents of Ringer's solution (section II) did not materially increase the life-span of double operated animals.

Study of the table and the various solutions employed, reveals the interesting fact that almost any solution when administered in large quantities is effective in prolonging life. For example, in section VII we find an average prolongation of 152.2 hours for the animals treated with sodium chloride, and in section VIII the data show that sodium bicarbonate is only slightly less effective, giving an average survival of 147.3 hours.

Certain solutions proved more efficacious than others in preserving life. Thus, solution 1, section III of the table, proved very effective, whereas solution 1-B, section V, and sodium acetate, section IX, were ineffective. In the case of sodium acetate, however, the animals had great difficulty holding the material as it had an irritating effect upon the stomach.

Zwemer (1926) observed that 5 per cent solutions of glucose given orally prolonged the life of double operated animals an average of nine days, whereas the untreated controls succumbed within 60 hours. Glucose appears to be more effective than any of the substances employed in the present experiment.

It is not at all clear why forcing fluids should prolong the life of bilaterally epinephrectomized cats. It is evident from the work of Swingle and Zwemer that dehydration is one of the train of symptoms following adrenal removal in cats, and flooding the organism with fluids tends to temporarily relieve this symptom of adrenal insufficiency. The explanation which most readily comes to mind in this connection is that the forcing of fluids and the consequent diuresis flushes some toxic substance out of the bloodstream of the organism. However this may be, it seems evident that the forcing of fluid definitely prolongs the life of bilaterally epinephrectomized cats, but this action is merely palliative and not curative, since all animals so treated eventually succumb with typical symptoms of adrenal insufficiency.

We believe that the period of survival of our treated cats is not due to the time interval between the first and second operation, since this factor was carefully controlled. Animal 16, section VI of the table, in which 5 days elapsed between operations shows a survival period of 177 hours; while no. 17, subjected to the same treatment, and having an 8-day interval between the first and second operations, shows a survival of 144 hours. The same is seen in a comparison of animals 19 and 21, section VII as well as nos. 23 and 24, section VIII. Cases of the converse do occur in the tables, however, as in cat 20, which shows a survival of 91.5 hours with a 4-day interval between operations, as against no. 21, on the same treatment, with a survival of 187.5 hours and an interval of 7 days between the extirpation of the glands. With a reasonable interval between the operations, 5 to 10

days, there does not appear to be any relation whatever between this interval and the length of the survival period. There can be little doubt however, in view of the results of other workers, that the lapse of long intervals between operations tends to prolong the survival period.

As regards the total weight lost after the second operation, it appears to be more closely related to the weight of the animal than to any other factor. At any rate, the animals show no more marked losses in weight under one type of treatment than another. As a rule, the heavier animals with large deposits of fat showed the most marked losses, as would be expected.

SUMMARY

1. The oral administration of large quantities of fluid to bilaterally epinephrectomized cats prolongs life.

2. Solutions of certain substances (i.e., glucose, NaCl, etc.) are more effective than others in maintaining the animal in a normal condition.

3. The explanation why forcing fluids prolongs the life of double operated cats is not entirely clear. It seems to partially relieve the dehydration which is one of the train of symptoms of adrenal insufficiency in cats, and possibly by the consequent diuresis flushes toxic substances out of the organism.

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AN EXPERIMENTAL STUDY OF THE ADRENAL CORTEX

I. The Survival Value of the Adrenal Cortex

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Seventy-seven years ago, Addison (1849) in speaking before the South London Medical Society, stated that he had at least an inkling as to the function of "these hitherto mysterious bodies—the suprarenal capsules." He described a peculiar type of anemia, which was associated with general weakness and an indisposition toward physical and mental exertion, and which terminated with death from exhaustion in a few weeks or months. In the cases that were brought to necropsy there was found a diseased condition of the suprarenal capsules.

In spite of the more than three quarters of a century that have elapsed since he first described this syndrome, we are still without a clear understanding of the rôle of the suprarenal glands in Addison's disease, or as to their function in the normal organism. Recent experimental work indicates that the cortex of the suprarenal is necessary for life and that the medulla of the gland may be removed without serious consequences resulting.

Stewart and Rogoff (1917), (1919) removed one adrenal and sectioned all the nerves joining to the other and found that after five weeks no adrenalin could be demonstrated in blood taken from the adrenal vein. They performed this experiment on dogs, cats, rabbits and monkeys, and their test was sensitive up to 1:1000 millions. It would seem quite evident from their data that adrenalin is not necessary for life, as their animals developed no abnormal symptoms.

Wheeler and Vincent (1917) in experiments on 30 dogs, 22 cats and 4 rabbits, removed one and one-half adrenals and cauterized the medulla from the remaining half. Three weeks to a month later the cauterized gland was removed post mortem and examined for medullary tissue. The examination showed that in "several" cases no medullary tissue was present. While not conclusive, this is further evidence for the importance of the cortex. Houssay and Lewis (1923) curetted out the medulla from one adrenal and removed the other a week or ten days later. Of the twenty-five dogs used a large majority survived until used for some other experiment. Only two animals were brought to a third operation, at which the

curetted gland was removed. The dogs lived for 24 to 26 hours after the removal of this cortical remnant, which had kept them alive for 217 and 232 days respectively.

Wislocki and Crowe (1923), using two dogs, removed all of one adrenal and two-thirds of the other, cauterizing the medulla in the part left in the animal. In addition they removed the abdominal chromaffin body. This procedure left the animals a negligible amount of chromaffin tissue and one-sixth of the original amount of cortex. The animals lived, showing no symptoms until the bit of cortex was removed.

Elman and Rothman (1924) secured results the reverse of this, by ligating the lumbo-adrenal veins and blunt dissection of the small blood vessels of the adrenals. They found that one gland hypertrophied and the other atrophied, the atrophied gland being mostly medullary tissue. The removal of the hypertrophied gland caused death, in spite of the presence of normally staining medulla in the other gland.

We have adopted the technique used by Houssay and Lewis (1923) and have secured essentially the same results with cats, as we showed in a preliminary report (Zwemer, 1924). In our series a larger number of animals were subjected to the final operation at which the surgically produced cortical gland was removed. Death followed its removal, showing that it was responsible for the maintenance of life.

The writer takes this opportunity to express his gratitude to Dr. W. W. Swingle, who so willingly gave encouragement and advice in the carrying out of these studies, and to the Biological Laboratory, Cold Spring Harbor, L. I., N. Y., for facilities during the summer of 1924.

EXPERIMENTAL. Cats were used in all our experiments because infrequency of accessory glands in these animals would tend to make the results more uniform, and it is well known that adrenal extirpation in cats leads to a fatal issue. In more than 100 animals used, only one was found with an accessory gland of cortical material. Moreover, cats have been used extensively in this laboratory for parathyroid work, and their care and feeding have been carefully studied. For animals that remained in the laboratory a long time, (i.e., series C) the standard diet of meat (cooked and raw) and milk was supplemented by fish.

The duration of life after the complete removal of the adrenals depends on the species used, and to a large extent on the time interval between the first and second operations (Elliott, 1914). For the last named reason, the interoperative period should always be given. Fairly uniform results can be secured with a wait of one to two weeks between operations. In all our tables this time interval is listed in the column immediately following the series number and sex of the cat.

The lumbar pathway was used for epinephrectomy in all the experiments, the adrenals being removed in two stages, one to two weeks apart.

Stewart (1924) has stressed poor technique as a factor in the rapid death of epinephrectomized animals, and discredited much of the early work, on the ground of injury to the animals. In earlier work (Stewart and Rogoff, 1917, 1919) they have shown that sectioning of the splanchnics prevents adrenalin from being secreted into the blood stream. On the other hand, they and Marshall and Davis (1916) found that cats showed no signs of adrenal insufficiency when the splanchnic nerves were out and one adrenal removed. We have found that removal of one gland has no noticeable effect on cats, and in series C the severe handling of one, and the removal of the other gland had no serious effect, provided the blood supply to the cortical tissue left in the animal, was not interfered with to too great an extent. However, we believe that much of the early work can be ruled out on the grounds of faulty technique, especially in those cases in which the animals still retained one adrenal, or died shortly after the removal of both adrenals, without having recovered their full activity for a time.

The chief measure of the efficacy of adrenal removal in cats, is the duration of life after the removal of the second gland, since even a small amount of cortex, if left, is sufficient to maintain life (Biedl 1913; Wislocki and Crowe, 1924). As the value of our work depends to a great extent on the duration of life after complete epinephrectomy, we present an unusually large series of control animals, from which we were able to compute the usual postoperative period in cats deprived of their adrenals. The control operations were performed throughout the entire three years of experimental work, so as to meet the varying conditions of the seasons.

A. Effect of total removal of the adrenals from normal cats. Table 1 shows two series of controls, Z and S. The cats in series S have a slightly shorter survival average than series Z, but this is no doubt due to the fact that they were used for certain blood tests, and the extra handling of the animals and loss of blood would provoke an earlier death.

All of the animals listed in table 1 were not watched during the actual terminal period, but enough of them were, to give a picture of the general occurrences, and these coincide with the observations of previous investigators. In cases in which the animal was found dead, allowance was made in computing the duration of life, this depending on the condition of the body when found.

The removal of the second adrenal at first seems to have no effect. The animal recovers in a short time from the effects of the anesthetic, and for 24 hours seems normal. In many cases (Z 3, 5, 9, 14, 17) the cats drank milk, and in others (Z 1, 4, 18, 19) even ate meat after the operation.

An early symptom is the refusal of food, first solid and then liquid. Most of the animals will drink water until a few hours before death, but not in sufficient quantity to maintain their weight. The weight loss in these control animals averages about 4 per cent of their body weight and

TABLE 1

Life span and weight changes of epinephrectomized cats (control series Z and S)

NUMBER	SEX	DAYS BETWEEN OPERATION I AND II	SURVIVAL IN HOURS AFTER OPERATION II	INITIAL WEIGHT	WEIGHT AT DEATH	WEIGHT CHANGE
		days	*hours*	*grams*	*grams*	*grams*
Z1	♀	7	68	2540	2490	−50
Z5	♀	7	111			
Z9	♀	11	45			
Z14	♀	11	47			
Z15	♀	9	39			
Z17	♀	8	68	2960	2770	−190
Z18	♀	7	68			
Z19	♀	7	51			
Z2	♂	7	70	2720	2610	−110
Z3	♂	10	26			
Z4	♂	7	67			
Z8	♂	7	62			
Z13	♂	25	55			
Z16	♂	7	48	2490	2100	−390
Z20	♂	12	51	2290	2130	−170
S1	♂	9	27	2940	2850	− 90
S2	♂	20	48	3615	3450	−165
S3	♂	18	43	2810	2660	−150
S4	♂	5	55	3110	3095	− 15
S5	♂	8	41	2135	2055	− 80
S6	♂	10	45	2310	2115	−195
S7	♂	6	46	2735	2580	−155
S8	♂	5	59	3410	3280	−130
S9	♂	3	77	2630	2380	−250
S10	♀	13	55	3175	2985	−190
S11	♂	7	52			
S12	♂	5	60			
S13	♂	11	52			
S14	♂	6	50			
S15	♂	8	48			
S16	♂	10	24			
S17	♂	10	50			
S18	♂	4	58			
S19	♂	6	30			
S20	♂	5	48			
S21	♂	7	37			
S22	♂	7	50			
S23	♂	5	72			
S24	♂	13	57			
S25	♂	13	51			
S26	♂	11	75			
S27	♂	13	36			
S28	♂	2	48			

occurs mainly after the first 24 hours following total epinephrectomy, and may be due to lack of food, rather than a result of adrenal insufficiency.

Concurrent with the refusal of food, there is a lack of desire to play with the rest of the animals, the cat preferring to remain quiet. This general lassitude is followed by muscular asthenia, which is first evidenced in the hind legs and later in the fore legs. In spite of this weakness the animal can be aroused to great activity, but sinks to even greater lethargy when the stimulation is removed. The muscular weakness is followed by depression, the animal sinking into a typical position the head on the forepaws and the hind limbs stretched out to the side as shown in figure 2. In the later stages the animal is flat on its side (as in fig. 3,) and either goes in to coma (Z 10, 18, 19) or may have a spasm resembling the "Jacksonian march" with all the extensors coming into play (Z 4, 5, 9). Respiration is rapid until the premortal stage is reached, and then slows down to as low as ten a minute. Some animals emit a sort of cry or moan with each expiration, but this does not always occur. An early symptom is that of lowered temperature of the extremities. This coldness is most easily determined by feeling the ears, which in normal cats are quite warm, but in epinephrectomised animals are cold to touch. The heart goes on beating for a while after respiration ceases, and in a few cases was still beating when the thorax was opened at necropsy. One heart was excised and placed in warm Ringer's solution and kept on beating spasmodically for about an hour after the excision. Other general observations were that the skin becomes gray, dry and wrinkled and loses its elasticity. When the cat is picked up in the usual manner by the back of the neck, the skin remains in a fold for some time. The mucous membranes are dry and lustreless.

Our observations of symptoms following epinephrectomy in cats correspond in most respects to those made by Elliott (1914), Marshall and Davis (1916), and Moore and Purinton (1901). Other investigations have shown that removal of the adrenals causes a rise in the total blood solids (Donath, 1916), doubles the normal amount of urea in the blood (Marshall and Davis, 1915) causes anoxemia and plasma concentration (Gradinscu, 1913) lowers the blood sugar and increases the erythrocyte count (Stewart and Rogoff, 1925).

In view of these findings, and the fact that the symptoms noted agree to a remarkable extent with those of anhydremia as given by Mariott (1923), it would appear that there is a loss of the liquid constituents of the blood. This point will be taken up again in another paper.

Necropsies were performed on all animals, to ascertain whether death was due only to the removal of the adrenal glands. When an examination of the organs showed some pathological cause for death, the case was discarded. Severe infections, when present, were sufficient to rule out a case. Animals that lived longer than usual, were carefully examined for acces-

sory glands, and no suspicious tissue was allowed to escape careful scrutiny and in many cases histological examination.

The average survival time in our control series of 43 cats was 53 hours, the extremes being 24 hours (S 6) and 111 hours (Z 5). This average duration of life after total epinephrectomy serves as the chief criterion in the work that follows, in which we have attempted to determine the survival value of the cortical portion of the adrenal complex following the removal of the medulla.

B. The relative importance of the cortical portion of the adrenal complex for the maintenance of life. Operative procedure: This involves three stages, which in table 2 are referred to as operations 1, 2 and 3.

The first step is the production of a cortical gland by surgical means. One adrenal, preferably the left as that is easier to handle, was exposed by the lumbar approach. Instead of removing the gland, its blood supply was temporarily clamped off with a curved hemostat. It was then opened longitudinally with a sharp razor blade, and the medulla spooned out with a small curette. To make the removal of all the medulla doubly certain, binocular dissecting microscope was used to inspect the gland within the body, and any remaining bits of the grayish medulla, together with a considerable portion of the reticular layer of the cortex were removed. The gland was then sutured with two or three stitches, the clamp on the blood vessels released, and the body wall closed.

A week to ten days later the other adrenal was removed intact, and symptoms observed. If the remaining cortex had not been damaged too severely at the initial operation, the animal recovered, with no signs of adrenal insufficiency.

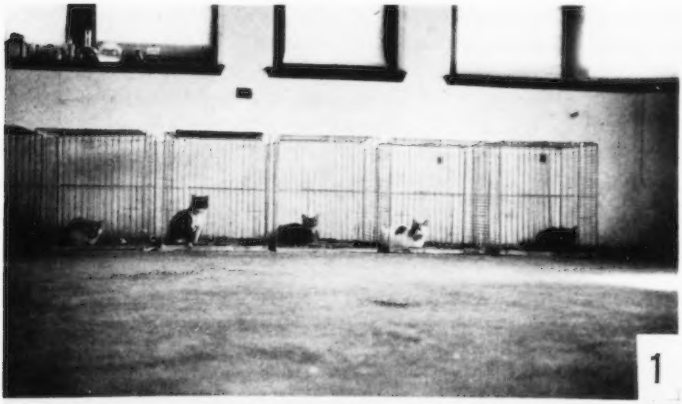
Three weeks to six months after the second operation, if the animal had developed no unusual symptoms, the surgically produced cortical gland was removed. This third operation is more difficult than the other two, due to the presence of scar tissue and the establishment of a collateral blood supply. In spite of the severity of the operation, the cats survived an average of 4.8 days after the removal of the remaining cortex, the shortest survival being one day and the longest eight days. The fact that the animals in this series survived total epinephrectomy for a longer time than the controls, can be accounted for in the light of Elliott's (1914) work,

Fig. 1. Operated animals in their cages. Three of these animals retain only the cortex of one adrenal.

Fig. 2. Shows the second cat from the right in figure 1 in typical position assumed by epinephrectomized cats shortly before going into coma.

Fig. 3. The same animal in coma, with two cats still retaining the cortex of one gland.

Fig. 4. Two animals, retaining only the cortex of one adrenal gland, shown fighting.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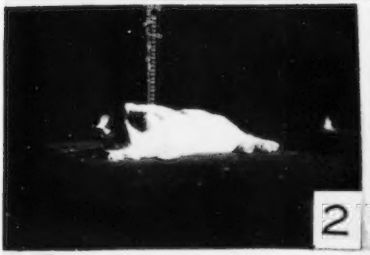
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and our own data. He found, and our work confirmed his findings, that the survival period is lengthened if three weeks or more are allowed to elapse between operations. In all our cases in this series the wait between the second and third operations was at least three weeks.

An examination of table 2 shows the fate of the animals subjected to the above mentioned operations. The time interval between operations is given and also the survival period after the third operation (cortex

TABLE 2

The survival value of the adrenal cortex (series C)

NUMBER	SEX	DAYS BETWEEN OPERATION I AND II	DAYS BETWEEN OPERATION II AND III	SURVIVAL IN DAYS AFTER III OPERATION	INITIAL WEIGHT OF ANIMAL	WEIGHT CHANGES BETWEEN OPERATION I AND II	WEIGHT CHANGE BETWEEN II AND III	WEIGHT CHANGE BETWEEN III AND DEATH
		days	*days*	*days*	*grams*			*grams*
*C1	♀	11	17	—	2060			−715
C3	♀	8	(12)					
C4	♀	7	(15)					
C5	♀	7	(23)		1200	+10		−520
C6	♀	7	(12)		1150	−140		−130
C7	♀	(21)			930			−40
C8	♀	(27)			1130			−60
C10	♀	7	25	5	4200	−120	+370	−450
C11	♀	7	26	7	3795	−10	+65	−300
C12	♀	7	173	1	2770	+5	+345	−105
†C13	♀	8	23	9+	2385	+125	+180	+20
C15	♀	7	21	6	2030	+40	+20	−180
C16	♀	7	24	2	2505	+25	+55	−275
*C17	♀	7	29	—	3170	−30	−190	—
C18	♀	7	33	3	2875	−30	+565	−255
C19	♀	8	38	8	3840	−50	+760	−680
C20	♀	10	(20)	—	4125	−25		−1115
C21	♀	12	2	—	2375			−235
C22	♀	10	40	2	3005	+180	−65	−120

* C1 and C17 died on the operating table at the third operation.

† C13 was the animal that failed to show symptoms after the removal of the surgically produced cortical gland. On being brought to necropsy, it was found to have an accessory gland of pure cortical tissue.

removal). The weight gains and losses in grams are also given, as well as the original weight of the animal.

One animal (C 1) died on the operating table at the third operation. An examination of the surgically produced cortical gland showed it contained only cortical tissue, thus demonstrating that removal of medulla was possible.

Another animal (C 21) lived only 2 days after the second operation. This may have been due either to the removal of too much cortical material,

or an occlusion of the blood supply at the initial operation. Four kittens (C 3, 4, 5, 6) survived the removal of the medulla of one adrenal and the entire other gland, for a considerable period. Two lived 12 days, one 15 days, and one 23 days. Two more kittens (C 7 and 8) had only the first operation performed on them, and died after 21 and 27 days, while still retaining one gland and the cortex of the other. However, Elliott and Tuckett (1906) have stated that kittens succumb after the removal of a single adrenal. One adult cat (C 20) showed a gradual onset of adrenal symptoms after the removal of the second adrenal. It survived for 20 days. The curetted gland, on sectioning, showed a complete degeneration of the two inner layers of the cortex, and only a small area of the zona glomerulosa was present. This case and that of the four kittens show a gradual production of adrenal symptoms by operative means.

Lacassagne and Samssonow (1923) and Wislocki and Crowe (1924) produced gradual insufficiency symptoms in rabbits and dogs, by implantations of radium in one adrenal and removal of the other. The longest prolongation in their cases was 32 days. Marine and Bauman (1921) produced insufficiency symptoms in rabbits after two weeks, by freezing the glands with ethyl-chloride spray.

Our case C 20 is remarkable in that it recovered from the second operation for a day, as is usual, and then had a period of depression for five days, showing transient adrenal insufficiency symptoms. During this period it drank a great deal of water, but would not take food. It then gradually recovered and was quite active for eight days, but soon began to show signs of muscular weakness and dehydration. Six days after the second onset of symptoms it died in convulsions, which were preceded by a five-hour period of coma. Its weight loss was very great, 1115 grams in 20 days, which was well over 25 per cent of its original weight of 4125 grams.

Ten animals of this series recovered from the second operation and in a very short time could not be distinguished by their actions, from the normal cats in the laboratory, although retaining only the cortex of one adrenal. After an interval of three weeks to six months (see table 2) the surgically produced cortical remnant was removed. Death ensued after an average interval of 4.8 days, the cats showing all the usual symptoms of adrenal insufficiency.

Earlier mention was made of the fact that during the period between the second and third operations, while the animals had only the cortex of one gland, they behaved normally, and ate as much as normal cats, played, fought, and even copulated, as if nothing at all had been done to them. These animals are shown under varying conditions. Figure 1 is a picture of them in their cages in the animal room. Figure 4 was taken out on the roof where the animals were allowed to run. The cage was used to keep the fighting animals within range of the camera. The one on its hind legs

is C 13, the other C 12. Figures 1, 2 and 3 give the story of C 11. In figure 1 it is seen in the second cage from the right, in 2, it is in the typical posture of cats dying of adrenal insufficiency, the head resting on the paws. In 3, it is in coma, and near by are animals C 15 and C 13. In figure 1 it retains the cortex of one gland, and in figures 2 and 3, none. The striking contrast between the animals with and without cortex can be easily seen in figures 2 and 4.

One very notable case (C 13) showed absolutely no symptoms after the third operation and the supposed removal of all its remaining cortical tissue, and after nine days this animal was killed and carefully autopsied. An accessory adrenal was found, the only one in all of the cases listed in this paper. It was found posterior to the kidney and located on the spermatic vein. There were a number of small vessels all leading into the capsule. Figure 5 shows the location of this gland, and also the usual position of the adrenals. This accessory on sectioning, proved to be of pure cortical material, and thus furnishes a natural check on the experimentally produced cortical glands (see fig. 6).

Table 2 gives, in addition to the time intervals between the various operations, and the survival period after the third or final operation, the weight changes that occurred in the animals of this series (C).

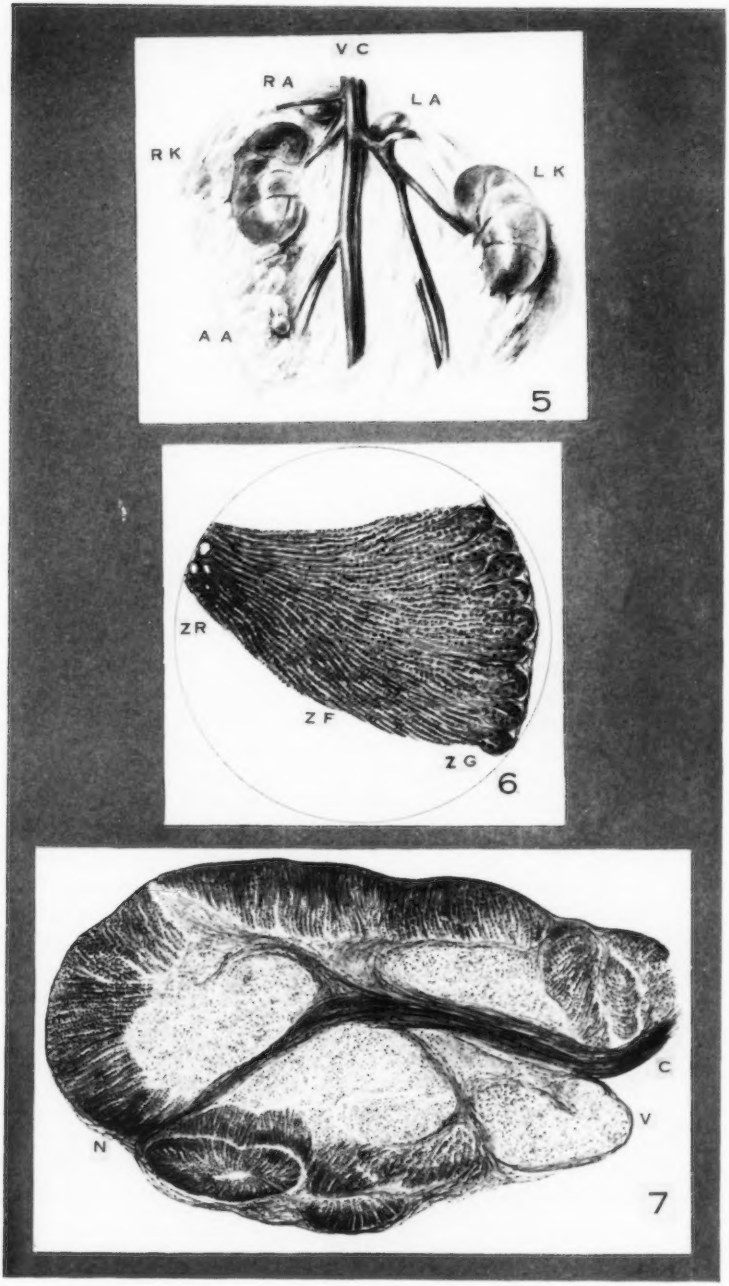
It would appear that the weight loss of animals dying of adrenal insufficiency symptoms depends on the length of time they survive after the removal of all their adrenal tissue, or, in some cases, on the interval between the onset of symptoms and death. One animal, C 19, lost 608 grams in the 8 days that it survived, while C 12, surviving only 1 day, lost 105 grams.

The glands removed at the third operation were preserved and sectioned in order to determine whether or not any medulla had been left behind after the gland had been scraped. For a quick diagnosis in the earlier cases, the frozen section technique was used, and this also proved to be the best in showing the state of the gland and the chromaffine tissue when present. This was obtained by fixing the gland in Muller's fluid-formalin, which stains functioning medulla the characteristic orange-yellow color. The glands were then examined without staining, by sectioning when frozen, and mounting the sections in glycerine. The parts of the gland not

Fig. 5. Drawing showing the position of the accessory cortical gland found in cat C13, and the normal position of the adrenals in relation to the vena cava and the kidneys. *AA*, accessory gland; *RA*, right adrenal; *LA*, left adrenal; *VC*, vena cava; *RK*, right kidney; *LK*, left kidney.

Fig. 6. Section through the accessory gland of pure cortical material. *ZG*, zona glomerulosa; *ZF*, zona fasciculata; *ZR*, zona reticularis.

Fig. 7. Camera lucida drawing of a section through an adrenal from which the medulla had been curretted out, 33 days previously. The central area shows the scar formed after the operation, and some degenerate cortex. *N*, normal cortical tissue; *V*, vacuolated cortical cells; *C*, connective tissue in scar.


VC
RA
LA
RK
LK
AA
5
ZR
ZF
ZG
6
C
V
N
7

used for this determination were later used for making sections by the paraffin method cut at 10μ. Delafield's hematoxylin used alone did not obscure the chromaffin reaction, but other stains were also used, to determine that no chromaffine tissue was present.

Of the ten animals (C 10, 11, 12, 13, 15, 16, 17, 18, 19, 20) that survived the third operation and had the retained cortex sectioned, only two showed medullary tissue distinguishable histologically and giving the chromaffine reaction. C 10 showed about 12 cells in a mass of connective tissue, and C 16 a small clump of cells that gave the chromaffine reaction and two smaller groups that were of the same structure, but did not show any chromogen granules. From the above study we determine that eight animals, having only the cortical tissue of one adrenal, lived for three weeks to six months, without showing any signs of adrenal insufficiency, and that on removal of this cortical tissue, they, with one exception, died showing typical symptoms of adrenal insufficiency. The exception, on being brought to autopsy, was found to have an accessory gland, composed of pure cortical material, and with a copious blood supply (figs. 5 and 6).

From this evidence, we believe that the cortical portion of the adrenal complex is the part essential for life in cats, and interference with its blood supply, or its removal from the animal causes the symptoms that have been attributed to the adrenal as a whole. The medulla, at least that within the adrenal complex, is not essential for life or the activity of the animal.

C. The survival value of transplants of the adrenal cortex. Having determined the vital function of the adrenal cortex, the next experiment sets forth an attempt to make functional autografts of adrenal cortex, in the hope that the grafts would maintain the life of animals deprived of their adrenals.

Autoplastic grafts of the endocrine glands have been made using a variety of forms. In mammals, it has been indicated that the gonads, and the parathyroids can be successfully transplanted. A review of grafting experiments of the gonads is given by Lipschutz (1923) and the parathyroids by Morel (1912) and Swingle and Nicholas (1925). Transplantation of the adrenal, however, has not met with much success.

Most, if not all workers, attributed their lack of success to the fact that in their grafts, the medulla rapidly degenerated. In those cases in which success was reported, medullary tissue was always present. It was our belief, from a study of the relative value of the cortex and medulla of the adrenal, that possibly the presence of the medulla interfered with the success of the graft. The following experiments show an attempt to secure the survival of epinephrectomized cats, by means of functioning grafts of the adrenal cortex.

Operative procedure: At the time of the second operation, the remaining adrenal was removed, cut in half longitudinally and the medulla removed.

One-fourth or one-half of the gland was then placed in a pocket prepared for it, either in the rectus abdominis muscle, or beneath the tunica vaginalis of the testis. The latter site was chosen because of Stilling's (1905) success in being able to recognize cortical cells after having been grafted into the testes for three years. A few grafts were tried in the peritoneal cavity, the graft being held in place with sutures.

The peritoneal grafts were made at the time of the first operation, on the assumption that perhaps the time interval between the operations would be enough to allow a blood supply to be established but not long enough for the graft to be resorbed. The grafts into the muscle and testes, being made at the time of the second operation, had to supply an immediate demand. Both types of grafts were equally unsuccessful, in so far as permanent substitution for the whole gland was concerned, but the survival value of the cortex was shown in a prolongation of life, in those cases in which the graft "took."

From an examination of table 3 it can be seen that in five cases the animals derived no benefit from the graft and died within 3 days (B 4, 8, 16, D 4, 5). Of the animals that did live longer than the usual survival period, B 15 lived 4 days, B 2 and 20 lived 5 days. Two of these animals were pregnant and one recently delivered. Of the remaining eleven animals, two lived 6 days, seven 7 days, one 8 days and one 9 days. Counting only the animals that received some benefit from the grafts we get an average survival of $6\frac{1}{2}$ days, which is more than twice as long as that of the control series.

The progress of the insufficiency symptoms of an animal deprived of its adrenals, and with a transplant of cortical material, was much more gradual than that of the controls.

Weight changes in this series were only taken on animals with peritoneal transplants, so may not be significant for the whole series. The changes for the individual animals can be seen in table 3. The transplanted cats lived twice as long, and lost twice as much weight as the control, or completely epinephrectomized animals. However, these animals generally did not eat for 4 or 5 days before death, and starvation may be the factor in the weight losses.

The histological changes of the grafts are not given in tabular form, because they were the same in all cases. The completeness of the degeneration depended on the length of time the graft was in the animal. The sequence of events was: fat formation in the cortical cells; disappearance of cell structure, i.e., nuclei in a vacuolated cytoplasmic mass; necrosis, followed by fibrillar connective tissue invasion.

The results of these experiments, while negative in the production of a functioning graft, are encouraging in that the survival period of animals deprived of their adrenals can be more than doubled by transplants of the

adrenal cortex. The microscopical examination of the transplants showed clearly that no growth had occurred. Therefore, we believe the prolongation to have been due to a resorption of the cortical tissue, and a utilization of the hormones contained in it at the time of implantation. In any event, the fact that grafts of adrenal cortex prolong the life of animals

TABLE 3

The prolongation effect of transplants of the adrenal cortex (series B and D)

NUMBER	SEX	DAYS BETWEEN OPERATION I AND II	SURVIVAL IN DAYS AFTER OPERATION	INITIAL WEIGHT	WEIGHT AT DEATH	WEIGHT CHANGE
a. Grafts in the peritoneal cavity						
				grams	*grams*	*grams*
D2	♀	7	7	3410	3190	−220
D3	♂	7	8	3330	3010	−320
D4	♂	7	2	2490	2200	−290
D5	♀	8	3	2960	2770	−190
b. Grafts in the testes						
B4	♂	25	2	Previous infection		
B14	♂	7	7			
c. Grafts in the rectus abdominis muscle						
B2	♀	7	5	Pregnant		
B3	♀	21	7	Pneumonia		
B5	♀	19	6	Pneumonia		
B6	♀	10	7			
B7	♀	10	9	Slight infection		
B8	♀	11	2	Pneumonia		
B10	♀	8	7	Pregnant		
B12	♀	8	6	Infection		
B13	♀	5	7	Infection		
B15	♀	7	4	Pregnant		
B16	♀	9	2	Pneumonia		
B18	♀	7	7			
B20	♀	14	5	Recently delivered		

Note: During the course of the transplantation experiments feline pneumonia broke out among the animals. This accounts for the incompleteness of the series and probably had some effect on the duration of life after complete epinephrectomy.

deprived of their adrenals, is further indication of the vital function of the cortical portion of the adrenal complex.

Discussion. That the adrenal glands are essential for life has been proved by many investigations. The few exceptional cases on record have been shown to be due to the presence of accessory adrenals in the species

used. Dogs, cats and guinea pigs rarely survive decapsulation, usually dying within a few days of the removal of the glands.

The survival period after epinephrectomy has been used as a criterion in a study of the adrenal complex in the cat. In 43 animals, this averaged 53 hours. The time interval between complete removal of the adrenals and death, depends to some extent upon the time interval between the removal of the first and second adrenal (Elliott, 1914). Stewart and Rogoff (1925) have been able to secure longer survival periods in dogs, than any previous workers. They attribute the rapid onset of symptoms described by previous workers as due to the effects of the operation, rather than to the removal of the adrenals. Undoubtedly the severity of the operation has something to do with the hastening of death after removal of the glands. However, in series C of the present studies, equally severe operations were performed as on the controls, and the animals recovered and were to all appearances normal until the removal of the surgically produced cortical gland. The unilateral operation seems to have no ill effects on the animal, regardless of whether the left or right gland is removed.

The results of the experiments on the survival value of the cortical portion of the adrenal, give further and more complete evidence as to the relative importance of the medulla and the cortex. Cats showed no symptoms of adrenal insufficiency so long as they retained the cortex of one adrenal. Removal of this surgically produced cortical gland induced typical adrenal insufficiency symptoms in the animals, leading to death in a few days. A larger number of animals than have been heretofore reported, underwent the crucial third operation, at which time the cortical remnant was removed.

Evidence for the importance of the cortical portion of the adrenal has been given by Biedl (1913), Wheeler and Vincent (1916) and Houssay and Lewis (1923). This work led to the present studies. Additional evidence for the predominant position of the cortical portion of the adrenal, is offered by the work of Lacassagne and Samssonow (1923), Wislocki and Crowe (1924) and Elman and Rothman (1924).

The evidence for the importance of the medulla offered by Vassale and Zangfroginni (1902) and Vassale (1905) must be disregarded, since their animals died while still retaining one adrenal. They used young cats in their experiments, and Elliott and Tuckett (1906) have shown that kittens succumb after the removal of a single gland.

A. and H. Cristiani (1902) also claim that some medullary tissue is necessary for life, but as they used rats, 60 per cent to 80 per cent of which have accessory cortical glands, their work cannot be taken into account.

The only conclusion possible, from the accumulated data, is that the cortical portion of the adrenal complex is essential for life, and that symp-

toms of adrenal insufficiency are due to removal of the cortex and not the medulla of the gland.

Our experiments on transplantation of the cortex only, were unsuccessful in producing a functional graft, but showed that the presence of the cortical tissue was of some benefit in that it prolonged the life of epinephrectomized cats to more than double the usual survival period. This prolongation, the writer thinks, is due to a resorption of the cortical tissue and its contained hormone, no additional hormones being elaborated by the graft.

It seems a surprising thing that autografts of the cortex do not "take," as autografts of nearly every other tissue are capable of growing in some other part of the same body. The fact that cortical cells persist for some years, but do not sustain life, would indicate that in their case some essential factor was lacking for the proper functioning of the implanted tissue.

SUMMARY AND CONCLUSIONS

1. Unilateral extirpation of the adrenal of cats has no ill effect on the animal.

2. Cats deprived of both adrenals, in two stages with an interval of from 5 to 20 days between operations, will survive on an average, 53 hours; the extremes being 26 and 111 hours.

3. Animals retaining only the cortex of one adrenal with undisturbed blood supply, survive indefinitely, showing that the medullary portion of the adrenal complex is not essential for life.

4. The removal of this surgically produced cortical gland causes the death of the animal in from one to eight days. Histological examination showed that the glands were composed only of cortical tissue. Therefore we conclude that the cortex is the portion of the adrenal complex essential for life in cats.

5. An accessory gland composed of pure cortical material was found to have sustained the life of one epinephrectomized animal.

6. Transplants of the adrenal cortex prolonged life in epinephrectomized cats for an average of $6\frac{1}{2}$ days, but degeneration of the graft eventually caused death.

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AN EXPERIMENTAL STUDY OF THE ADRENAL CORTEX

II. Prolongation of Life after Complete Epinephrectomy

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A. The effect of the thyroid on animals deprived of their adrenals. Earlier investigators have reported changes in the adrenals after thyroidectomy and thyroid feeding, and have shown a correlation of some sort between the two glands. Cannon (1916) found that the adrenal hypertrophied after the thyroid secretion had been augmented by electrical stimulation of the latter gland. Other investigators (R. G. Hoskins, 1910; E. R. Hoskins, 1916; Herring, 1917) observed that thyroid feeding caused the adrenal to hypertrophy in rats. Herring (1916) also found that thyroidectomized adult male rabbits had smaller adrenals than normal. On the other hand, Carlson (1916) stated that thyroidectomy in rabbits causes the adrenal to hypertrophy to two or three times the normal size. This seeming inconsistency can be explained by the work of Gley (1914). He found that thyroidectomy in rabbits produced hypertrophy but not hyperfunction. There was, rather, a fatty degeneration of the cells. This observation is further evidence that the functional activity of an endocrine gland cannot be determined by its weight or size. The relative size of the thyroid in endemic and exophthalmic goitre is another striking demonstration of this fact. In spite of this evidence, some biological statisticians insist that a functional interrelation of endocrine glands can be proved by correlations of weight or size.

Evidence as to the interrelation of the thyroid and the adrenal in metabolism has been offered by Cameron (1923), Marine and Baumann (1922), and Marine, Baumann and Cipra (1925). The latter believe that the adrenal cortex secretes a metabolism depressing substance, since feeding adrenal cortex lowers heat production, and also counteracts the effects of thyroid feeding.

In the following experiments, of which a preliminary report has already been made (Zwemer, 1925) the duration of life, after complete removal of the adrenals, has been taken as a criterion, in a study of the effect of thyroidectomy and thyroid feeding on epinephrectomized animals.

The writer takes the opportunity to express his obligation to Dr. W. W.

Swingle for suggesting this problem and for advice in carrying out the experiments.

Operative procedure. 1. Thyroid absence. In the first four animals listed (series A) the thyroids had been removed about three weeks before the adrenals were removed. The latter were taken out by the lumbar route in two stages.

Eight more animals (series E), were treated as follows: The first time the animal was anesthetized the thyroids and left adrenal were removed. The parathyroids were at the same time dissected out in a medium of sterile Ringer's solution and blood serum and replaced in the neck as grafts, in order to prevent the onset of tetany (Swingle and Nicholas, 1925). At the second operation the right adrenal was removed.

As all of these animals were kept under the same conditions of feeding and living quarters as the controls described in part I of these studies, it can be seen that any variation in the duration of life after the removal of both adrenals would be due to the absence of the thyroids. A study of table 1 shows that the absence of the thyroids tends to delay the onset of adrenal insufficiency symptoms, and to prolong the life of an animal so treated.

The table also gives the interoperative period, weight changes, and the duration of life after thyroid and epinephrectomy. In eleven of the twelve cases there was a considerable prolongation of life. The one exception died of tetany several hours after the second operation, due to failure of the parathyroid graft. It had shown slight tetany symptoms for 9 days previous to the removal of the remaining adrenal and had been relieved by administration of 5 per cent calcium lactate orally.

The longest survivals were 14 days (E 4) and 22 days (A 2), the other cases grading between them, and cat E 7 lived only 3 days. This short lived case was complicated by the fact that it tore open its neck wound. The average survival period including all eleven cases is over eight days (200 hours). This is nearly four times the survival period of epinephrectomized animals still retaining their thyroids, which averaged 53 hours.

2. Thyroid feeding. In order to determine the converse of the above experiments it was necessary to have an additional type of control. These were normal unoperated cats that were fed a definite amount of thyroid per day, merely to see whether it would induce any symptoms in them. Two cats of this series (Y) were fed 4 grains of desiccated thyroid per day for one week, two others were given the same dosage for two weeks. The effects noted were an increased activity and appetite and a decrease in weight. One animal (Y 2) gave birth to a litter of kittens during the course of the feeding experiment, and that accounts for the apparent great decrease in weight, in her case (see table 1).

TABLE 1

The effect of the thyroid on epinephrectomized cats

a. Thyroidectomy (series A and E)

NUMBER	SEX	DAYS BETWEEN OPERATION 1 AND 2	SURVIVAL IN DAYS AFTER SECOND OPERATION	INITIAL WEIGHT	WEIGHT CHANGES BETWEEN OPERATION 1 AND 2	WEIGHT CHANGES BETWEEN OPERATION 2 AND DEATH
		days	*days*	*grams*	*grams*	*grams*
A1	♂	20	6			
A2	♂	19	22			
A3	♀	10	7			
A4	♂	7	6			
E1	♀	12	8	2600	−100	−250
E2	♂	12	8	3000	+100	−510
E3	♂	12	(1 hour)	3100	−660*	
E4	♂	12	14	3200	−90	−310
E5	♂	13	7	3620	−220*	−290
E6	♀	13	5	2860	−50	−280
E7	♀	8	3	2170	0	−170
E8	♀	11	6	2310	+20	−240

b. Thyroid feeding (series F)

NUMBER	SEX	DAYS BETWEEN OPERATION 1 AND 2	SURVIVAL IN HOURS	INITIAL WEIGHT	WEIGHT CHANGES BETWEEN OPERATION 1 AND 2	WEIGHT CHANGES BETWEEN OPERATION 2 AND DEATH	AMOUNT OF THYROID FED
		days	*hours*	*grams*	*grams*	*grams*	*grams*
F1	♀	12	12	2060	−250	−30	24
F2	♂	5		2695	(−105)*		
F3	♀	8	15	2000	−300	−10	24
F4	♂	12	21	2910	−180	−30	36
F5	♀	10	18	2480	−230	−50	32
F6	♂	15	23	3330	−505	−25	52
F7	♂	9		3910	(−200)*		20

c. Normal, unoperated animals fed thyroid as a control (series Y)

NUMBER	SEX	WEIGHT BEFORE FEEDING	WEIGHT AFTER FEEDING	WEIGHT CHANGE	AMOUNT OF DESICCATED THYROID FED, AND DURATION OF EXPERIMENT
		grams	*grams*	*grams*	
Y1	♂	2800	2610	−190	56 grains in 14 days
Y2	♀	2650	1970	−680†	56 grains in 14 days
Y3	♂	3100	2980	−120	28 grains in 7 days
Y4	♀	2710	2550	−160	28 grains in 7 days

* E3 and F2 developed severe tetany and died in tetany convulsions. E5 and F7 showed transient tetany symptoms.

† Cat Y2 gave birth to kittens on the sixth day of the experiment, thus accounting for the increased weight loss.

Having determined that feeding thyroid had no severe effects on unoperated animals in the dosage given, this same daily amount of thyroid was given to cats that had been epinephrectomized. The thyroid was also removed, to obviate any complications that might arise due to the thyroid taking up and holding inactive, part of the thyroid feed. The operative procedure was the same as that given above for the thyroid absence type of experiment, and conditions for the experiments were the same, the only difference between the two series being that in the second series, the cats were fed 4 grains of desiccated thyroid per day between the first and second operations.

Seven animals were used in these feeding experiments (series F, table 1). In every case the animal died within 23 hours after the removal of the remaining adrenal, the average for this series being 18 hours. This is in great contrast to the average survival of 200 hours, of animals having the same glands removed, but not fed thyroid. We would emphasize the fact that epinephrectomized animals without thyroids live, on an average, 200 hours; with their thyroids 53 hours; and when fed thyroid only 18 hours.

This rapid death of the thyroid fed animals cannot be attributed to faulty technique, because the same procedure was followed for all the series of animals, and these animals recovered from the operation and walked around, drank milk and in other ways demonstrated that the operative procedure had not affected them in any way. Necropsies in every case showed nothing unusual.

Of the seven animals used, five survived until the second operation (F 1, 3, 4, 5, 6); one (F 2) died of tetany the fifth day after the first operation; the remaining animal (F 7) died of adrenal insufficiency symptoms although still retaining one gland. This case was complicated by the occurrence of transient tetany on the third and fourth day after thyroidectomy, but it gives an indication, together with an earlier case of a kitten (not listed) deprived of both thyroids and one adrenal and fed thyroid, that in some cases, one adrenal may not be sufficient to counteract the effects of excessive thyroid feeding.

The evidence would tend to show that the thyroid has a marked effect on the duration of life of cats after epinephrectomy. Two possible explanations of this effect will be given in the discussion, but in view of the results of the following experiments involving the oral administration of glucose solution and a study of the weight changes, we believe it to be a dehydrating effect of the administered thyroid.

B. The relief of adrenal insufficiency symptoms by the oral administration of glucose solution. Observations on all the animals dying of adrenal insufficiency, showed that many of the symptoms coincided with those of anhydremia. There is a rapid weight loss; the skin becomes gray, dry and wrinkled, and loses its elasticity (as shown by the dry fold when an animal

is picked up); the mucous membranes become dry and lustreless; the extremities are cold, although the rectal temperature may be high; the respirations in the pre-coma stage, rapid and stertorous, indicating air hunger.

An attempt to relieve this condition by the oral administration of water was unsuccessful. In the two cases attempted, we failed to get prolongation of life and included them in our list of control animals (Z 18 and 19).

Failing to get prolongation with water alone, we next tried the oral administration of glucose solution, hoping the glucose would perform the double feat of assisting in the retention of the water and correcting the marked hypoglycemia which invariably follows bilateral epinephrectomy.

Six animals were used in this experiment. Each of them was given 100 cc. of 5 per cent glucose solution by stomach tube, three times a day. This

TABLE 2

The effect of oral administration of glucose solution on the life of epinephrectomized cats (series G)

NUMBER	SEX	DAYS BETWEEN OPERATION 1 AND 2	SURVIVAL IN DAYS AFTER SECOND OPERATION	INITIAL WEIGHT	WEIGHT CHANGE BETWEEN OPERATION 1 AND 2	WEIGHT CHANGE BETWEEN OPERATION 2 AND DEATH
		days	*days*	*grams*	*grams*	*grams*
G3	♂	8	11	3400	+10	−140
G4	♂	7	6	2200	+20	−390
G5	♂	8	8	2110	+20	−160
G6	♂	7	9	2950	+25	−380
G7	♀	13	8	2550	−10	−170
G8	♂	13	10	2270	+5	−165
G9	♀	10	5	1270	+30	−120

G4 and G9 were not included in computing the average survival as they both developed slight stitch infections, and any infection tends to shorten the survival period of epinephrectomized cats.

was done immediately following complete epinephrectomy, which was done in two stages, the time interval between operations being 7 to 13 days.

The symptoms of adrenal insufficiency appear the fourth day after the second operation, and the onset of symptoms is very gradual as compared with the controls. The average duration of life in these animals, omitting the two having infections, was 9 days, or 220 hours.

If the animals could have been induced to eat, or if food had been given them by stomach tube, they might have survived a much longer time, but in order to be sure that it was the glucose solution that was prolonging life, nothing else was given until the fifth day after the second operation. Then, in four cases (G 3, 4, 5, 6), 1 gram of peptone and 2 cc. of cod liver oil were added to 100 cc. of the glucose solution. This was omitted in cases G 7 and G 8 as it seemed to have no beneficial effect on the animal.

So far as the writer is aware, this is the first successful attempt to prolong the life of animals deprived of their adrenals, by the oral administration of glucose or any other solution.

There are two papers in the literature that indicate that the introduction of liquid into epinephrectomized animals is beneficial. Soddu (1899) reported slight alleviation of symptoms by bleeding dogs and then injecting them intravenously with saline solution. Stewart and Rogoff (1925) found that intravenous injections of Ringer-Locke-glucose solution greatly prolonged the life of dogs deprived of their adrenals. Some of their animals survived as long as 32 days, the intravenous injections being given at intervals of a few days. No symptoms of insufficiency were observed until about 48 hours before death.

Aub, in a very brief abstract (1925), stated that glucose had no effect on the symptoms of adrenal insufficiency in cats. In the same abstract he gives 33 hours as the average survival time for his series of control animals, and claims to have prolonged this to 55 hours, by the administration of extracts of the adrenal cortex. Both of these periods are within the range of our control periods (average 53 hrs.), and for this reason the prolongation seems too short to be significant.

Hartman (1926) reports an average prolongation of life of epinephrectomized animals to 146.6 hours, with a saline extract of the cortex.

Mention has been made of the success of Soddu (1899), Stewart and Rogoff (1925) and the writer (1925) in prolonging the survival period of animals deprived of their adrenals. Corey in this laboratory (in print) has also found that a large variety of salt solutions, when given orally, will greatly prolong the survival period of animals deprived of their adrenals. Some of the effects of cortical extracts mentioned in literature may be due to the introduction of considerable amounts of liquid into the animals dehydrated by epinephrectomy.

The effects of the thyroid, and of the oral administration of glucose solution can also best be explained on the basis of dehydration especially in the light of recent work by Swingle (1926) of this laboratory. The work of Swingle, which is the first complete study of the changes in the blood and urine of epinephrectomized cats, indicates some of the causes back of the dehydration phenomena. A possible explanation of this, and the foregoing experiments with the thyroid, will be taken up in the discussion.

Discussion. The experiments showing the relation of the thyroid to the life of animals deprived of their adrenals shed further light on the rôle of the adrenal cortex in the organism.

In the absence of the thyroid, epinephrectomized cats survived an average of 200 hours, whereas the average survival of the control animals, that still retained their thyroids, was 53 hours. On the other hand, the

feeding of thyroid, thus giving the animal an excess of the hormone, shortens the average survival period after epinephrectomy to 18 hours.

If the rôle of the adrenal cortex is that of a detoxicating agent, as many investigators assume, the explanation of the thyroid effect would be that, in the absence of the thyroid, metabolism is slowed down and fewer toxic metabolites are formed. The feeding of thyroid would have the opposite effect of greatly accelerating metabolism, thus increasing the possible formation of toxic substances.

A more probable explanation is offered in considering the thyroid as a dehydrating agent. Eppinger (1917) found that in edema due to myodegeneration of the heart, or to various types of nephritis, thyroid administration causes a discharge of fluid from the body. On the other hand, after thyroidectomy elimination of fluid is much delayed. Cherfils (1926) reports similar effects after thyroid administration in a number of clinical cases of nephritis. Loeb (1923) thinks it probable that the thyroid substance acts primarily on the intermediary distribution of salts and water, rather than on the kidney. It has long been known that in marked cases of myxedema, due to atrophy or hypofunctioning of the thyroid, rapid loss of water occurs shortly after administering thyroid material.

Since the symptoms of animals dying of adrenal insufficiency coincide in a most striking manner with those of anhydremia, the removal of the thyroid, which is known in certain cases to act as a dehydrating agent, may have delayed the onset of fatal symptoms by slowing down the elimination of water from the organism. Conversely, it is possible that feeding of thyroid substance for a considerable period, caused an early death after removal of the adrenals, due to the rapid elimination of water. The loss in weight of these animals between the first and second operation (table 1) favors this explanation.

The relief of adrenal insufficiency symptoms by oral administration of glucose is striking. The average prolongation was 220 hours. This is over four times the usual survival period of epinephrectomized cats.

Reports of relief of adrenal insufficiency symptoms in dogs, by intravenous injections, have been given by Soddu (1899) and Stewart and Rogoff (1925). Rowntree (1924) of the Mayo Clinic used intravenous injections of glucose to relieve shock occurring in cases of Addison's disease (as much as 600 cc. of a 10 per cent solution).

Recent work by Swingle in this laboratory (in print) indicates that one of the underlying factors back of the dehydration of epinephrectomized animals is an acidosis. He found a marked fall in the pH of the blood of cats from which the adrenals had been removed. With this fall in the pH there is a rise in the inorganic phosphates and an accumulation of organic acids. It is known that dehydration phenomena are associated with acidosis.

SUMMARY AND CONCLUSIONS

1. Feeding of thyroid substance to unoperated normal cats, causes a decrease in the weight of the animal. This is believed to be due to the dehydrating effect of the thyroid, and to the increased metabolism.

2. The removal of the thyroid glands prolongs the life of animals deprived of their adrenals to an average of 200 hours. The absence of the thyroid apparently tends to decrease the elimination of water, and the symptoms of adrenal insufficiency, which strikingly resemble those of anhydremia, are postponed.

3. An excess of thyroid substance, produced by daily feeding of the desiccated gland, during the interval between the removal of the first and second adrenal, hastens the death of epineprectomized animals. The thyroid appears to increase the elimination of water from the organism, thus adding to the dehydration effect following adrenal extirpation. The animals survive on an average only 18 hours after the removal of the second adrenal.

4. The oral administration of a 5 per cent solution of glucose, if given in sufficient quantities, will prolong the life of epinephrectomized cats for an average of 220 hours.

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STUDIES ON THE FUNCTIONAL SIGNIFICANCE OF THE SUPRARENAL CORTEX

I. Blood Changes Following Bilateral Epinephrectomy in Cats[1]

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Numerous investigations have appeared within the last few years concerned with the function of the suprarenal glands. The problem of the functional significance of these structures has been attacked from various angles, but to date the rôle played by the adrenals within the organism remains an unsolved mystery. Despite the sacrifice of many animals, and a vast amount of labor, the cause of death following ablation of these glands is unknown.

The researches of Biedl (1913), Wheeler and Vincent (1917), Wislocki and Crowe (1924), Houssay and Lewis (1923), and Zwemer (1925–26) in this laboratory, show conclusively that the portion of the adrenal which is essential for life is the cortex, since destruction of the medulla is without demonstrable effect, whereas severe injury to or removal of the cortex results in typical symptoms of adrenal insufficiency terminating in death.

Certain changes in the blood of mammals following adrenal removal have been reported from time to time, but so far as the writer is aware no systematic study of the blood chemistry of bilaterally operated animals has been made. It was our original intention to make a thorough study of all of the blood constituents, but as the work progressed certain phases proved especially interesting and worthy of further consideration, and so were studied in detail, thereby necessitating a curtailment of the original program.

Cats were chosen for experimental animals chiefly because they rarely possess accessory adrenals, and bilateral epinephrectomy is usually followed by death within a short period. Zwemer (1926) working in this laboratory, found that cats which have had their adrenals removed at two

[1] The expenses of this investigation were defrayed by a grant from the Bache Fund of the National Academy of Sciences. The writer is indebted to the Biological Laboratory, Cold Spring Harbor, N. Y., for laboratory facilities during the summer of 1926.

stages five to ten days apart rarely live more than sixty or seventy hours. The present writer found that the average survival of forty-nine bilaterally operated cats (interval of seven days between operations) was sixty hours. All of these animals were operated during the fall and winter months. Cats which died within the first eighteen hours were excluded since it is probable that other complicating factors besides adrenal removal may have shortened the survival period. Since this work was begun, the writer and his students have bilaterally epinephrectomized over two hundred animals and have perfected an operative technique which is simple and involves practically no shock to the animal. Only carefully selected cases were employed for blood analysis, and cats which had colds, stitch infections or any symptoms not usually associated with suprarenal insufficiency were excluded.

The writer takes this opportunity of expressing his obligation to Miss Carmen Rothwell of the Pediatrics Laboratory of the Yale Medical School, and to Miss Phyllis Stanley of the Presbyterian Hospital, Newark, N. J., for assistance in carrying out the blood analyses.

The following methods were employed: sodium, Kramer and Tisdall (1921); phosphorus, Briggs' modification of the Bell and Doisy method (1922); calcium, Kramer and Tisdall (1921); non-protein nitrogen, Folin and Wu (1919); total nitrogen, Kjeldahl; blood sugar, Folin and Wu (1919); acetone, Van Slyke (1917). Potassium and magnesium determinations were also made but with such inconstant results that the figures are not included.

The left adrenal gland was first removed from the animal and five to seven days allowed to elapse before the right gland was extirpated. Unilaterally operated cats do not show symptoms of adrenal insufficiency if the remaining gland is normal. Two cats out of two hundred cases under observation at this laboratory died with typical symptoms following removal of one gland. In both cases autopsy revealed a degenerate and atrophic right suprarenal—the degeneration involving both cortex and medulla. However, it has been our experience that unilaterally operated cats remain normal, hence all of our control blood analyses were made on animals with one gland removed. The blood was obtained by cardiac puncture while the animals were under ether (with the exception of blood for sugar determinations) and was obtained a few minutes before the remaining adrenal was removed. The second blood was taken at varying times following the onset of marked adrenal symptoms but without ether. The blood for sugar determinations was obtained by first gentling the animals and quickly drawing blood from the heart before the cats had time to struggle. The symptoms of adrenal insufficiency in cats have been described in detail by Elliott and Tuckett (1906) and Zwemer (1926) so need not be repeated here.

Phosphorus. One of the most striking and consistent changes in the blood constituents following adrenal removal is the rise in the serum phosphorus. In most cases the increase is considerable, especially in the pre-coma stage. The accumulation of phosphorus appears simultaneously with the development of serious symptoms. Animals in coma or prostrate in the pre-coma stage invariably show a sharp increase in blood phosphorus. On the other hand, decapsulated animals which show but slight symptoms of adrenal insufficiency, such as lethargy and beginning weakness, have phosphorus values but little above the normal range. It is possible to use the phosphorus level of the blood as a prognostic sign—high phosphorus invariably indicating that coma and death are but a few hours distant.

The phosphorus values for unilaterally operated cats appear to be somewhat variable, ranging from 4.5 mgm. per 100 cc. of blood to 9.8 mgm. The phosphorus level in the double operated cats ranged from 5.9 mgm. to 16.3 mgm. So far as the writer is aware, no one has reported changes in blood phosphorus following adrenal extirpation. The significance of the increase in phosphorus will be discussed later in connection with changes in kidney function. The increase in phosphorus is not to be regarded as solely a terminal or premortal event, because the increase appears before the extreme symptoms are present. Nor is the phosphorus increase to be accounted for on the basis of increased blood concentration. It is true that the blood of doubly operated cats does show concentration but the following experiment indicates that blood concentration is not responsible.

Cat XY was doubly operated and developed symptoms of adrenal insufficiency such as loss of appetite, weakness of the hind legs, and lethargy. The animal was bled at 5 p.m. and then injected with 100 cc. of Ringer's solution. The following morning at 9 a.m. the cat seemed somewhat brighter and was bled again. The animal finally died in coma at 9 p.m. of the same day. The results of the two blood analyses are shown below.

Before Ringer's solution

Phosphorus	5.8 mgm.
Calcium	9.4 mgm.
Nitrogen	1.15 grams
Protein	7.2 grams
Non-protein nitrogen	41.4 mgm.

After Ringer's solution injected

Phosphorus	6.9 mgm.
Calcium	9.0 mgm.
Nitrogen	0.99 gram
Protein	6.2 grams
Non-protein nitrogen	40.4 mgm.

TABLE 1

Changes in the blood following epinephrectomy

CAT	SEX	UNILATERALLY OPERATED ANIMALS								BILATERALLY OPERATED ANIMALS							
		P	Ca	Na	Protein	Total nitrogen	Non-protein nitrogen	Urea	Symptoms	P	Ca	Na	Protein	Total nitrogen	Non-protein nitrogen	Urea	Symptoms
		mgm.	*mgm.*	*mgm.*	*per cent*	*per cent*	*mgm.*	*mgm.*		*mgm.*	*mgm.*	*mgm.*	*per cent*	*per cent*	*mgm.*	*mgm.*	
1	♂	7.2	9.9	342	8.8	1.41	42.0		None	12.2	9.8	331	9.7	1.49	91		Verging on coma
2	♀	6.1	9.5	339	7.5	1.25	43.0		None	9.2	9.8	328	8.6	1.32	68		Verging on coma
3	♂	4.5	10.2	405	7.5	1.25	44.8		None	5.9	8.6	324	5.7	1.02	97		Very weak. Shock*
4	♀	7.7	9.4	345	7.3	1.22	46.5		None	8.2	9.2	323	7.1	1.22	77		Very weak. Shock*
5	♂	9.5	11.0		8.2	1.31			None	15.2	12.1		8.6	1.37			Coma
6	♂	6.5	10.3		8.2	1.31			None	13.0	11.4		9.2	1.47			Weak
7	♂	9.8	8.4		8.0	1.28			None	15.1	9.7		8.5	1.37			Coma
8	♂	8.4	10.4						None	12.3	12.8						Very weak
9	♂	6.1		312					None	11.6		310					Very weak
10	♂	8.1		335					None	16.3		330					Coma
11	♂	9.2		336					None	13.4		329					Coma
12	♂	6.2	10.5						None	9.4	11.2						Verging on coma
13	♂	4.9					43.5	16	None	8.9					170	122	Coma
14	♀	6.8					44.0	26	None	10.6					76	51	Weak
15	♂						44.0	24	None						64	39	Weak
16	♂						49.0	22	None						91	60	Weak
17	♂				5.8		44.5	19	None				7.1		96	63	Weak
18	♂				6.4		41.0	21	None				7.1		67	40	Weak
19	♂				7.3		42.5	23	None				8.4		89	57	Very weak
20	♂				6.2				None				7.5				Weak

* Animals 3 and 4 died of shock and not from adrenal insufficiency.

A comparison of the two bloods taken before and after Ringer's Solution was injected shows that the degree of blood concentration does not account for the phosphorus rise, because the phosphorus continued to rise despite the fact that the blood had undergone dilution, as indicated by the decrease in protein and total nitrogen. Twelve hours after the second blood was obtained the serum phosphorus had risen from 6.9 mgm. per 100 cc. to 10.4 mgm. per 100 cc. (table 1, animal no. 8).

Calcium and sodium. The changes in the serum calcium following adrenal removal are not of any great significance. In general the calcium rises slightly after the second operation. In view of the fact that the serum phosphorus rises it was considered likely that the calcium would be decreased below the normal level, since several investigators have demonstrated that an inverse ratio exists between the levels of phosphorus and calcium under certain conditions. Barlow and Ellis (1924) cite a single case of an epinephrectomized cat in which the serum calcium rose from 10 mgm. per 100 cc. in the normal animal to 17 mgm. per 100 cc. following double adrenal removal. The blood specimen was taken shortly before death. None of our cases showed increases as great as this. Table 1 shows that the level of the serum calcium of the unilaterally operated cats ranged from 8.4 mgm. to 11 mgm. per 100 cc. of blood, whereas the doubly operated animals showed ranges from 8.6 mgm. to 12.8 mgm. per 100 cc. The slight increase in the serum calcium can best be accounted for as being due to increased blood concentrations.

Only a few sodium determinations were made since it soon became evident that this substance remained practically unchanged after adrenal extirpation. In general a slight drop in the serum sodium occurred (table 1) but the decrease is not of sufficient magnitude to warrant serious consideration. We did not anticipate any marked change in the sodium content of the blood since the level of this substance is not known to vary greatly in any pathological condition (table 1).

Non-protein nitrogen. The non-protein nitrogen always shows a sharp rise following double epinephrectomy, and at times the increase may be very great (table 1). The average value for non-protein nitrogen in unilaterally operated cats is between 42 and 47 mgm. per 100 cc. of blood, whereas the average amount found in the blood of double operated animals is about 91 mgm. Like the serum phosphorus, the non-protein nitrogen is always highest when the animals is in or verging on coma. The increase becomes apparent within the first twenty-four hours after operation and slowly increases until the animal enters the pre-coma stage when a sharp rise occurs. The highest figure for non-protein nitrogen recorded in our data is 170 mgm. per 100 cc. (cat 13, table 1). With the exception of this animal, the data presented in table 1 show that none of our figures are excessively high. The non-protein nitrogen values

for uremia, for instance, are far beyond anything encountered in bilaterally operated cats. In such cases the non-protein nitrogen may be as high as 360 to 400 mgm. per 100 cc. of blood. On the other hand true cases of uremia may show values approximating those of our bilaterally operated cats. It should be recalled that for the first eighteen hours following bilateral epinephrectomy, cats remain practically normal, and that the entire train of symptoms leading to death from adrenal insufficiency occurs within the next twenty to thirty hours. Apparently sufficient hormone from the adrenal cortex circulates in the blood to maintain the animal in a fairly normal condition during the first eighteen hours after double operation. Considering the short length of time between the second operation and death (about 60 hours) the increase in non-protein nitrogen is rapid.

Urea. The data on urea are not so extensive as could be desired, since but seven cases were studied (table 1). However, these cases are sufficient to show that the blood urea rises rapidly following double adrenal removal. This is of course to be expected considering the consistent rise in non-protein nitrogen which occurs.

There are other cases in the literature where increases have been reported in cases of adrenal insufficiency. Marshall and Davis (1916) reported that an increased concentration of urea occurs in the blood of cats following adrenal extirpation. They state that in cats which survive for several days after removal of both glands, the blood urea rises shortly after the second operation and attains a concentration about double the normal value. It remains at this level until about twenty-four hours before death, when a further steady rise occurs.

Marshall and Davis also report that analysis of the tissues of two dogs which died as a result of adrenal extirpation showed the presence of about five times the normal amount of urea.

Porak and Chabanier (1908) report finding an increase in the urea content of the blood of epinephrectomized rabbits eight to ten hours following adrenal removal.

It is interesting to observe that Rowntree (1925) in the course of an investigation of Addison's disease reports that several of his cases had high blood urea. He says: "Renal function studies were made in twelve cases. The blood urea was 30 mgm. or more for each 100 cc. in ten, 50 mgm. or more in four, and between 90 and 100 mgm. in two. Each of the last two patients appeared to be in extremis at the time the determinations were made, but the increase in blood urea remained unexplained. In one, a fatal case, the increase in urea persisted, but in the other patient it dropped quickly as the patient improved and it became normal within a week."

The evidence seems quite clear that the urea content of the blood rises following lesions or removal of the adrenal glands.

Protein and total nitrogen. The total nitrogen of the serum shows an increase in bilaterally operated cats. Table 1 shows the total nitrogen determinations for seven animals. It will be noted that cats 3 and 4 do not show an increase following the second operation. In fact the total nitrogen of cat 3 dropped from 1.25 grams per 100 cc. to 1.02 grams per 100 cc., and cat 4 remained unchanged. The protocols of both of these animals reveal the interesting fact that they died within twenty-four hours following removal of the remaining adrenal. All of the other cases reported lived considerably longer. Cat 3 survived the second operation twenty-four hours and forty minutes, and cat 4 lived but eighteen hours. The short survival period following the second operation renders these two cases doubtful. This probability receives support from a study of the other blood constituents. The serum protein and total nitrogen of cat 3 show decided decreases whereas in all other cases dying from typical symptoms of adrenal insufficiency, protein and nitrogen increase. Non-protein nitrogen increased in both cases however. These two animals are included in table 1 because they offer excellent confirmatory evidence for the view that all animals dying within twenty-four hours after removal of the second adrenal should be discarded as unsatisfactory cases since other factors than adrenal insufficiency probably caused death.

With the exception of the two animals mentioned the percent of serum protein rises after adrenal removal although the increase is not so great as we would expect considering the apparent increase in blood concentration.

Blood concentration. The blood of double operated cats shows considerable evidence of water loss. It is concentrated, does not flow freely into the syringe, clots more rapidly than the blood of unilaterally operated animals and yields smaller amounts of serum. Barlow and Ellis (1924) found that the blood coagulation time in 17 doubly operated cats was consistently shorter than in normal or unilaterally operated animals, and became progressively shorter as the death point was approached.

Blood concentration in our cases is indicated by the increase in serum protein (table 1). This increase appears to be due to a decrease in the liquid portion of the blood rather than to an actual increase in protein. It is the writer's impression that the concentration of the blood of bilaterally operated cats presenting marked symptoms is greater than the figures for serum protein indicate. Hemoglobin and cell volume determinations will be made in the near future to test this point.

Stewart and Rogoff (1925) made blood examinations of double operated dogs and state: "It is common to find towards the end of the survival period, perhaps only on the day before death or on the day of death, that

the relative volume of the serum dropped sharply. This was not infrequently accompanied by a diminution in the conductivity of the serum." These authors, however, caution against laying too much stress upon the terminal concentration of the blood. They report increases in hemoglobin in double operated dogs.

The evidence seems to indicate that a marked concentration of the blood occurs in bilaterally epinephrectomized animals about eighteen to twenty hours before death. Diluting the blood by injections of saline or glucose revives cats verging on coma but the effect lasts only a few hours. We have repeatedly injected cats in coma or verging on coma with gum-acacia in saline solution to increase the blood volume and raise its osmotic pressure. Such injections occasionally bring about a striking amelioration of symptoms but the effect is temporary and lasts but an hour or two.

TABLE 2

Blood sugar changes in normal and bilaterally operated cats

	NORMAL	OPERATED	SYMPTOMS
	mgm. per 100 cc.	*mgm. per 100 cc.*	
Cat 1	100	40	Coma
Cat 2	106	60	Prostrate
Cat 3	111	60	Prostrate
Cat 4	100	66	Prostrate
Cat 5	112	40	Coma
Cat 6	110	50	Coma
Cat 7	114	66	Prostrate
Cat 8	112	55	Coma
Cat 9	117	40	Coma
Cat 10	115	60	Very weak
Cat 11	112	55	Very weak
Cat 12	120	45	Prostrate

Blood sugar. It has long been known that the blood sugar of epinephrectomized animals shows a marked drop, and the same thing has been repeatedly observed in cases of Addison's disease. The determinations for blood sugar given here were made on a different set of animals than those employed for the other blood analyses. This was necessary because a normal blood sugar determination cannot be obtained from an anesthetized animal. The control blood was taken from unoperated cats. The following table shows blood sugar changes in twelve animals before and after bilateral epinephrectomy.

In accordance with the results of other investigators, table 2 shows that a striking fall in blood sugar occurs following adrenal extirpation. The normal blood sugar for cats is 105 to 118 mgm. per 100 cc. The drop begins about twenty hours after recovery from the anesthetic and con-

tinues slowly until death supervenes. One feature about the blood sugar decrease proved especially interesting – namely, that the onset of the symptoms of lethargy and muscular asthenia coincided with the drop in blood sugar. It was repeatedly noted that cats which were beginning to show muscular weakness and giddiness when walking, had blood sugars between 75 to 80 mgm. per 100 cc. of blood, and as the sugar decreased the asthenia became more marked until finally when coma developed, the blood sugar had fallen to between 40 to 55 mgm. per 100 cc.

These observations led to the belief that the muscular asthenia and progressive fall in body temperature which are practically invariably present as part of the train of symptoms of adrenal insufficiency in cats are due in part at least to the decrease in blood sugar. It is not claimed that the fall in blood sugar is the primary cause of adrenal death—far from it—for we have repeatedly injected double operated cats exhibiting marked symptoms with glucose, and observed that death can and does occur with high blood sugar. However, the hypoglycemia is of sufficient degree to

TABLE 3

Blood sugar of insulin injected cats

	NORMAL SUGAR	UNITS INSULIN	HOURS	SYMPTOMS	BLOOD SUGAR
	mgm.				*mgm.*
Cat 44	115	15	4	Coma	70
Cat 49	112	15	5	Coma	50
Cat 50	110	15	5	Coma	45
Cat 51	114	15	4	Coma	60
Cat 54	125	15	5	Coma	50

account for the asthenia and fall in body temperature. The following experiment bears upon this point.

A series of five cats was injected with insulin in order to produce a condition of profound hypoglycemia, and the symptoms compared with those following adrenal removal. In both conditions the onset of muscular weakness and giddiness occurred when the blood sugar had fallen to below 90 mgm. per 100 cc. of blood. The animals became unconscious and developed convulsions when the blood sugar fell below 60. Death occurred when the sugar of the blood reached the level of 55 to 40 mgm. In both "insulin shocked" cats and animals showing marked symptoms of adrenal insufficiency, the body temperature fell and the blood showed signs of concentration. Table 3 shows the blood sugar level at which normal cats (half grown) reach the convulsive stage when injected with insulin.

It was stated previously that the hypoglycemia itself cannot account for death following adrenal removal, because bilaterally operated cats

injected with glucose invariably die sooner or later despite the high blood sugar. However, cats in deep coma or verging on coma can be revived to a striking degree by injections of glucose. Within an hour following subcutaneous injections of 100 cc. of 10 per cent glucose plus 10 units of insulin an epinephrectomized cat in coma can be revived to such an extent that it will sit up or even walk about and seem on the road to recovery. Such animals show a high concentration of blood sugar, but despite this generally die in convulsions within several hours. Hence it is evident that the low blood sugar alone is not sufficient to account for the death of epinephrectomized cats, although all the symptoms of muscular weakness and giddiness can be attributed to this cause, because in insulin injected animals weakness, giddiness and convulsions appear when the blood sugar reaches the same level at which similar symptoms occur in operated animals. If an insulin injected cat is prostrate and on the verge of coma when the blood sugar drops to 50 mgm. per 100 cc. of blood, and we find the same symptoms and same blood sugar level in epinephrectomized cats, it seems fair to draw the inference that in both cases the symptoms are probably due to the hypoglycemia. If this be granted the cause of death of the double operated animals still remains unexplained, because glucose injections exert only a temporary effect. What other factor or factors are responsible? An attempt to answer this question will be made following presentation of certain other data.

The urine. During the course of the work it was noted that on the day when death occurred or on the day previous, the cats passed little or no urine. Some animals showed complete suppression, others were able to pass small quantities up until a few hours of death. Comparison of the total amount of urine passed by unilateral and bilaterally operated animals shows that after the second adrenal is removed the quantity of urine is much diminished.

Cat 93. Right adrenal removed April 13, 3 p.m. Left gland removed April 15.
April 14 passed 57 cc. urine containing 0.35 mgm. acetone per cubic centimeter.
April 15 passed 44 cc. urine containing 0.27 mgm. acetone per cubic centimeter.
April 16 passed 22 cc. urine containing 0.18 mgm. acetone per cubic centimeter.
April 17 passed 6 cc. urine containing 0.33 mgm. acetone per cubic centimeter.
Animal died in convulsions at 7 p.m. April 17. No urine in bladder.
Cat 94. Right adrenal removed April 13. Left gland extirpated April 15.
April 14 passed 62 cc. urine containing 0.09 mgm. acetone per cubic centimeter.
April 15 passed 56 cc. urine containing 0.28 mgm. acetone per cubic centimeter.
April 16 passed 24 cc. urine containing 0.24 mgm. acetone per cubic centimeter.
April 17 passed 16 cc. urine containing 0.25 mgm. acetone per cubic centimeter.
Animal died in convulsions at 5 p.m. April 17. No urine in bladder.

It is obvious that the output of urine is decreased in double operated cats. This can be partially accounted for on the basis of lowered blood pressure, since blood pressure falls in double operated animals. How-

ever, it has been shown by Marshall and Davis (1916) that the excretory function of the kidney of bilaterally epinephrectomized cats is diminished even when a normal blood pressure is maintained. They observed that the ability of the kidney to excrete certain dyes was considerably lessened after adrenal removal.

The data show that the ability of the kidney to excrete acetone remains normal up until the time of death. Albumin occurs in the urine of the double operated cats showing marked symptoms, but the concentration is not great.

The kidneys. Because of the retention of phosphorus and non-protein nitrogen in the blood, and the presence of albumin in the urine, the kidneys were examined for pathological changes. The procedure was as follows: The left adrenal and left kidney were removed and the kidney cut into thin pieces and fixed in Zenker's fluid. Five to seven days later the right adrenal was removed with especial precautions not to injure the kidney. When the cat passed into coma about sixty hours later, it was killed and the remaining kidney preserved, sectioned and compared with the normal left kidney. Delafield's hematoxylin, and eosin, were used for staining the tissue.

Macroscopic examination showed little or no change in the appearance of the kidney of the double operated animal beyond a slight congestion. Histological examination revealed several slight pathological changes such as small hemorrhagic areas throughout the cortex and cloudy swelling of the tubule cells. A detailed account of the pathological changes found in the kidneys of the double operated cats will be reported later.

Discussion. The chief findings resulting from this study of the blood chemistry of bilaterally epinephrectomized cats are: increase in the serum phosphorus, accumulation of urea and non-protein nitrogen, decrease in blood sugar, concentration of the blood, diminished urine output, and presence of albumin.

A study of the acid-base equilibrium of bilaterally epinephrectomized cats, carried on simultaneously with the present work (Swingle and Eisenman, 1926) revealed several significant facts when taken in conjunction with the results of this study. There is always a fall in pH, carbon-dioxide tension, serum bicarbonate, and total acid; indications of an uncompensated, non-volatile acidosis which apparently is due to an increase of phosphoric and organic acids.

The data indicate that death following removal of the adrenal glands may possibly be due to either of two pathological changes in the organism, neither of which are necessarily connected, but either of which is of sufficient magnitude to result in death if left uncorrected. The hypoglycemia which

results from adrenal removal is, in cats, severe enough to reduce the blood sugar within sixty hours to the level at which cats become unconscious and develop convulsions. The sugar level can be raised by glucose injections, yet the animals die eventually. Hence it is obvious that the hypoglycemia is but one factor responsible for death. The second factor is the failure of the kidney to excrete acid phosphate in sufficient quantities, with the result that an acid intoxication slowly develops which eventually leads to death. It is obvious, on the basis of the viewpoint expressed here, why epinephrectomized animals eventually die despite the fact that the hypoglycemia has been corrected by glucose injections. The injections relieve the blood sugar, but do not prevent the piling up of phosphorus and organic acids in the blood, and the resulting acidosis remains uncorrected.

The type of acidosis which develops following adrenal removal is of a similar nature to the acid intoxication characteristic of the terminal stage of certain types of nephritis. In both conditions failure of the kidney to properly eliminate acids seems to be the cause of the acidosis. The data indicate that the adrenal cortex supplies some hormone necessary for the maintenance of normal kidney function, and also that this secretion of the cortex probably acts as a brake or check upon the secretory activity of the islets of Langerhans.

SUMMARY

1. The blood of unilaterally and bilaterally epinephrectomized cats was analyzed for phosphorus, calcium, sodium, proteins, total nitrogen, non-protein nitrogen, urea and sugar. Significant increases in phosphorus, protein, non-protein nitrogen and urea were found in the double operated animals. The blood sugar decreases to the level at which convulsions occur in insulin injected cats.

2. The blood becomes concentrated, thus accounting for the rise in protein and total nitrogen.

3. The urine output is diminished and albumin appears.

4. Slight pathological changes were found in the kidneys.

5. The suggestion is made that death following adrenal removal may possibly be due to either of two causes: hypoglycemia and acid intoxication, since both phenomena are invariably associated with adrenal insufficiency. The hypoglycemia can be corrected by glucose injection but the animal eventually dies with symptoms of acid intoxication.

6. The acidosis appears to be due to failure of the kidney to properly eliminate acids, and is of the same general nature as that occurring as a result of certain types of chronic nephritis.

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STUDIES ON THE FUNCTIONAL SIGNIFICANCE OF THE SUPRARENAL CORTEX

II. The Acid-Base Equilibrium of Epinephrectomized Cats[1]

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Previous work by the senior writer and his students (Zwemer, 1924, 1925; Corey, 1926; Swingle, 1926) has shown conclusively that the part of the suprarenal complex in mammals that is essential for life is the cortex. The medullary portion can be removed without ill effects resulting, but if the cortex is destroyed or badly damaged, the animal exhibits typical adrenal insufficiency symptoms, and dies within a few days. The earlier work of Biedl (1903), Wheeler and Vincent (1917), Houssay and Lewis (1923), Wislocki and Crowe (1924), likewise indicated the great importance of the cortex for life. The functional significance of this portion of the suprarenal is at present unknown.

Because of certain striking changes in the blood which invariably follow adrenal (cortex) extirpation in mammals (Swingle, 1926) and the fact that the animals present unusual changes in the respiration some hours preceding coma and death, changes indicative of marked air hunger, it seemed probable that acid intoxication was one of the train of symptoms leading to death from epinephrectomy. With the idea of testing this hypothesis, and gaining some insight into the nature of the acidosis, if such existed, the coöperation of the junior author was enlisted.

So far as the writers are aware, no one has attempted a complete and thorough investigation of the chemical and physical changes in the blood following suprarenal extirpation. A few scattered investigations have appeared dealing with the changes in one or other constituent of the blood, but scope of inquiry has been too limited. The present report does not claim to be a complete picture of the blood changes following adrenal removal in cats. We were unfortunate in our choice of experimental animals, since the cat is a difficult animal to bleed repeatedly without serious struggling. On the other hand, we have on hand in the Osborn Zo-

[1] The expenses of this investigation were defrayed by a grant from the Bache Fund of the National Academy of Sciences.

ological Laboratory, the protocols of over two hundred completely epinephrectomized cats, with which to compare those of the present experiments.

METHODS AND PROCEDURE. Arterial or venous blood was drawn from the heart into a Luer syringe containing albolene and transferred without exposure to air into centrifuge tubes containing oil. The tubes were stoppered and the blood was immediately centrifuged. The serum was then transferred without exposure to air into a sampling bulb containing mercury, whence samples could be withdrawn for analysis. The technique used for the treatment of the blood has been described in detail by Austin, Cullen et al. (1922).

Serum proteins were determined by analyzing $\frac{2}{5}$ to $\frac{7}{1\frac{1}{2}}$ cc. of serum (diluted) by the macro-Kjeldahl method. From this total nitrogen value, an average non-protein nitrogen figure was subtracted. The result was multiplied by 6.25 to convert it to protein. The average normal non-protein nitrogen value was obtained from a series of ten analyses of normal cats' whole blood. The values ranged from 38 to 60 and gave a mean of 47 mgm. per hundred cc. blood. The average non-protein nitrogen value for operated cats was obtained from eight determinations of whole blood non-protein nitrogen from operated cats. The values ranged from 41 to 170, with an average of 83 per hundred cc. of blood. Such average corrections are only approximations. The non-protein nitrogen analyses were done on whole blood and on different animals from those used in this study. Whole blood non-protein nitrogen tends to be higher than serum non-protein nitrogen. Furthermore, non-protein nitrogen values for cats seem to be extremely variable. However, sufficient material was never obtained to accomplish the determinations on this series of cats, and, therefore, our protein values are not entirely satisfactory, but they serve to give an idea of concentration changes. The error in the pre-operative determinations probably does not exceed 0.1 per cent; that in the post-operative may be as high as 0.5 per cent of protein.

Serum carbon-dioxide content determinations were made on 1 cc. samples according to the Van Slyke (1924) technique in the constant volume apparatus.

pH determinations were made by the gasometric method described in a previous communication by one of the writers (Peters, Bulger and Eisenman, 1926).

Serum chloride was determined by the Van Slyke (1923) method.

Inorganic phosphorus was determined by the Benedict-Theis (1925) method.

Total base was determined by a slight modification of the Stadie-Fiske (1925) method.

CALCULATIONS. The acid values of protein, bicarbonate, chloride and phosphorus were calculated as previously described (Peters et al., 1926).

Total acid was taken as the sum of all the determined acids.

Organic acid was calculated by subtracting total acid from total base, and, of course, includes sulphate ion.

EXPERIMENTAL. *Normals.* As normal animals, cats were used that had completely recovered from unilateral adrenalectomy. Such animals are perfectly normal and experience with over two hundred cats with one adrenal removed has convinced us that one adrenal is amply sufficient to maintain a cat in normal condition. The blood serum was analyzed for protein, carbon-dioxide content, sodium chloride, phosphorus, pH, and total base. The data are given in table 1. Eleven cats were bled. Eight were bled while under urethane anesthesia; three cats that gave external

TABLE 1

Unilaterally operated cats

CASE	NATURE OF BLOOD SPECIMEN	URETHANE ANESTHESIA	STRUGGLING	SERUM PROTEIN	SERUM PROTEIN	pH	CARBON DIOXIDE TENSION	SERUM BICARBONATE	SERUM CHLORIDE	SERUM PHOSPHORUS	TOTAL ACID	TOTAL BASE	ORGANIC ACID
				per cent	*mM.*		*mm. Hg.*	*mM.*	*mM.*	*mM.*	*mM.*	*mM.*	*mM.*
ZI	Venous	+	0	5.89	10.8	7.48	27.8	21.3	109.6	3.5	145.1	154.1	9.0
ZN	Arterial	+	0	6.40	10.9	7.30	35.2	18.0	115.9	4.0	148.7	161.3	12.6
XC	Venous	0	0	7.32	12.3	7.27	42.7	19.9	113.2	3.8	149.2	163.5	14.3
V	Venous	+	0	6.91	11.6	7.26	33.4	15.5	115.6	2.8	145.5	154.4	8.9
ZM	Venous	0	*	6.28	10.7	7.31	37.0	19.3	118.5	5.6	154.1	175.2	21.1
ZX	Venous	0	0	5.97	10.1	7.29	33.1	16.2	111.3	3.6	141.3	162.6	21.3
SA	Venous	0	0	6.16	10.5	7.31	38.0	19.7	109.4	5.2	144.8	173.1	28.3
SB	Arterial	+	0	6.46	11.5	7.41	33.0	21.6	106.8	3.5	143.3	186.4	43.1
ZK	Arterial	+	0	6.18	10.7	7.35	28.3	15.8	111.8	3.6	141.9	156.7	14.8
ZF	Arterial	+	0	7.78	13.6	7.37	31.0	18.4	103.1	2.5	137.6	146.0	8.4
ZE	Arterial	+	0	7.53	13.2	7.37	26.7	15.8	109.6	3.0	141.5	157.5	16.0

* Slight.

evidence of slight or no struggling were bled without anesthesia. The variations in the individual constituents were so considerable that it was impossible to obtain satisfactory average normal values. If the three specimens obtained without anesthesia are excepted, more concordance in values is observed. The three excepted specimens show high base and high organic acid and the pH is relatively lower than that observed in specimens obtained under anesthesia. These facts, we believe, offer internal evidence of muscular activity on the part of the animals, despite lack of external evidence, as the results are comparable to those of exercise in normal human individuals.

Of the anesthetized animals, SB shows high base and high organic acid. Inasmuch as the total base is extremely high—in fact greater than any

other "normal" total base value recorded, it may be assumed that the analytical value for total base and the consequent calculated value for organic acid are incorrect. The second blood, i.e., that following extirpation of the remaining adrenal was not obtained, the animal dying during the night.

Animals after double adrenalectomy. After the second adrenal had been removed, blood samples were obtained from animals exhibiting symptoms of varying severity. The serum was analyzed for the same constituents as that of the normals or unilaterally operated animals. The results are given in table 2. Certain differences from the normals are grossly evident:

(1) The serum bicarbonate content always drops.
(2) The tension of carbon-dioxide always drops.
(3) There is always a fall in pH.
(4) Proteins rise in all but one case (SA).
(5) There is usually a marked rise in phosphorus, which may become very high when the animals pass into coma (Swingle, 1926).
(6) Total acid always diminishes.

Certain values show variations:

(1) Changes of chloride concentration are variable.
(2) Total base shows a striking drop in three cases. (ZM, ZS and SA.)
(3) Organic acid is not consistently affected, although it tends to be higher in the operated animals.

Discussion. The drop in total acid is almost completely at the expense of bicarbonate. The fall in bicarbonate concentration and carbon-dioxide tension cannot be due to over-ventilation, because the latter would result in an increase of pH. Dyspnea and hyperpnea are invariably present shortly before the animals pass into coma and is one of the striking symptoms of adrenal insufficiency in the preterminal stages. (See protocols.) In these experiments, pH has fallen, indicating that the bicarbonate reduction is due to a non-volatile acidosis for which the respiratory mechanism has not compensated. Chloride concentration is not consistently or significantly altered, and, therefore, cannot be the cause of the carbon-dioxide change. Nor can increase in protein concentration be responsible, because it is offset by the decreased base-binding power of the proteins at the lower pH. The fall in bicarbonate can not be occasioned to any considerable extent by the rise in phosphorus, which although marked, never exceeds 2 millimols, while the carbon-dioxide falls from 6 to 10 millimols.

The increase in organic acid is the one change of sufficient magnitude to be responsible for the bicarbonate fall. In considering the possible causes for the rise of organic acid, ketosis cannot be excluded, since some of the

TABLE 2

Cats after bilateral epinephrectomy

CASE	NATURE OF BLOOD SPECIMEN	URETHANE ANESTHESIA	SYMPTOMS	PROTEIN	SERUM PROTEIN	pH	CO_2 TENSION MERCURY	SERUM BICARBONATE	SERUM CHLORIDE	SERUM PHOSPHORUS	TOTAL ACID	TOTAL BASE	ORGANIC ACID
				per cent	*mM.*		*mm.*	*mM.*	*mM.*	*mM.*	*mM.*	*mM.*	*mM.*
ZI	Arterial	0	Moderate	7.14	11.6	7.19	24.5	9.7	115.9	5.0	142.2	158.9	16.7
ZN	Venous	0	Coma, serious	7.10	11.3	7.14	30.5	10.5	112.8	6.2	140.8		
SC	Arterial and venous	0	No coma	8.42	13.2	7.11	30.7	10.1	116.2	4.8	144.3	164.4	20.1
V	Venous	0	Blood drawn under artificial respiration	7.34					114.4	5.2		155.9	
ZM	Arterial and venous	0	Coma, difficult bleeding						116.1	6.3		155.0	
ZX	Arterial	0	Mild	6.51	10.9	7.27	21.5	10.0	106.0	3.6	130.6	141.5	10.9
SA	Venous	0	Mild	6.11	10.2	7.26	25.5	11.7	111.1	5.2	138.3	160.0	21.7
ZO	Venous	0	Convulsions	6.97	11.0	7.13	31.7	10.9	108.2	6.0	136.1	154.8	18.7

animals received little or no food after the second operation.[2] In the two of the three cases where values for organic acid were obtained, the animal exhibited only moderate symptoms. The rise of organic acid in these cases may well be due, as in the case of the normals, to muscular activity, as no anesthetic was employed. In the single case with severe post-operative symptoms from which a value for organic acid was obtained, the animal unfortunately passed into convulsions, while bleeding was in progress. The high organic acid in this instance is, therefore, of especially doubtful significance.

In these post-operated animals, high organic acid is not, as in the normals, associated with high total base. The blood from these animals gives apparent evidence of concentration. It is viscous and hard to draw and relatively little serum is obtainable from the whole blood. If such concentration is due to dehydration, and such appears to be the case as shown by other work (Swingle, 1926), the reason for the low total base is forthcoming. Dehydration is usually associated with a loss of total base. The usual associated loss of chloride was, however, not observed in our operated animals.

Serum protein is usually used as a measure of blood concentration. Although a slight rise in proteins is observed in the post-operative specimens, this small increase does not correspond to the apparent concentration of the blood. Dehydration and blood concentration in these cases are possibly associated with an actual loss of protein from the serum, due to a changed permeability of the blood vessel wall. The discrepancy can hardly be referable to the errors in protein determinations discussed above.

If the values for the different components are corrected for inspissation of the blood in order to compare the actual amounts rather than the concentrations of these constituents in the blood before and after operation, the conclusions are not altered because the blood volume changes are so small.

The differences between the values for arterial and venous blood are so small that the results are not significantly affected if they are used interchangeably.

It is to be regretted that the data are so incomplete. More extensive experiments are necessary before any definite conclusions can be drawn as to the nature of the acidosis following complete extirpation of the adrenals. There can be no question but that a marked acidosis is one of the cardinal symptoms of the pre-coma and coma stage in cats, but we have no clue at present as to the nature of the acid or acids. Further studies should in-

[2] Since this was written, repeated determinations for acetone in the blood of comatose animals presenting indications of acidosis following adrenal extirpation, have shown that less than 3 mgm. per 100 cc. are present. Ketosis evidently is not responsible for the rise in organic acid.

clude besides serum non-protein nitrogen determinations to correct serum proteins,—hemoglobin (oxygen capacity) and cell volume measurements, in order to determine whether the blood concentration change is greater than is indicated by the change in serum protein values. The dog should be employed for experimental animal because of the large amount of blood needed for the various procedures.

Although data on these points are lacking in our own work, the investigations of several other workers indicate the results likely to be obtained. For examples, the serum non-protein nitrogen values (Swingle, 1926) are considerably higher in the completely epinephrectomized animals than in the unilaterally operated (normal) cats. Stewart and Rogoff (1925) have published some observations of the blood changes in epinephrectomized dogs such as hemoglobin, erythrocyte and leucocyte counts, conductivity of blood and serum with calculation of the number of cubic centimeters of serum in 100 cc. blood and the specific gravity of blood and serum. Their results indicate blood concentration of the double operated animals especially on the last day preceding death. These authors do not place too much stress upon the terminal concentration of the blood.

Rowntree (1925) states that he has observed several cases of individuals suffering from severe Addison's disease and which presented a shock-like condition, which seemed to show "blood concentration, as evidenced by its decreased water content and increased difficulty in obtaining venous blood. It is interesting to note also that two of the cases of Addison's disease described by Rowntree as in a shock-like condition showed a moderate acidosis." The acidosis was suspected on clinical grounds, and determination of the carbon dioxide capacity of the plasma revealed 31 and 36 volumes per cent respectively.

PROTOCOLS: *Cat ZM.* Small, gray, male weight 2830 grams. Right adrenal removed February 4, 4:30 p.m.; bled from the heart, February 14. No urethane given; venous blood obtained. Cat struggled only slightly. Left adrenal removed February 17, 3:30 p.m. Weight immediately after operation 2830 grams. Excellent condition February 20 at 12 m.; eats, plays and fights. Probably has accessory gland. Re-operated on the right side February 21. Region explored for accessory gland. Removed nodule of cortical tissue from the right side. Weight, 2760 grams, after operation. 8 a.m., February 22, typical adrenal symptoms at 2:30 p.m.; cat in coma; rapid, long-drawn respirations (37 per minute); bled with difficulty, blood greatly concentrated; 15 cc. of arterial blood obtained and about 30 cc. of venous; marked air-hunger present at time of bleeding. Died 3 p.m., February 22. The accessory gland sectioned; histological examination shows cortical tissue.

Cat ZN. Black and white, male. Right gland removed February 9, at 10 a.m. Bled from heart February 25, at 9 p.m. Six hundred milligrams urethane per kilo. Cat did not struggle. Left adrenal gland removed February 27 at 12 m. Weight after operation, 2807 grams. February 28, 9 a.m., symptoms present; marked weakness and drowsiness, 10 p.m., February 29. 5:30 a.m., animal in coma, unconscious; loud breathing; respiration 40 per minute; dilated pupils. Bled from the

heart; blood very concentrated. Marked air hunger. Weight at death, 2660 grams. This animal fed 40 cc. of milk by tube at 9 a.m., February 28.

Cat ZO. Large, black, male. Right gland removed February 14 at 10 p.m. Left adrenal removed February 17, 8 p.m. Animal fed 50 cc. milk 3 times daily. February 20 at 10 a.m., very bad symptoms; unable to walk; marked air hunger. Bled from the heart; venous blood. Cat went into convulsions when the blood was being withdrawn. First blood of this animal was not obtained.

Cat ZX. Gray, male. Left adrenal removed February 15, at 10 a.m.; bled from the heart at 9 a.m. No urethane given; venous blood obtained. Cat did not struggle. Right adrenal removed February 27 at 12:30 p.m. Weight, 2758 grams. February 28, 9 a.m., animal shows slight symptoms. 10 p.m., animal weak, but not in coma, or did not show marked air hunger. Symptoms slight. Bled from heart at 10 p.m. Animal did not struggle. Arterial blood obtained. Weight before bleeding, 2710 grams. Animal received 32 grams of milk, 8 p.m.

Note: At the time of the second bleeding the symptoms were not marked.

Cat SA. Small, white and black, male. Right gland removed February 27, 4 p.m. Bled from heart, March 4 at 12:30 p.m.; venous blood obtained. Cat did not struggle. No urethane given. Left adrenal removed March 7, 11:50 p.m. Weight, just before operation, 2135 grams. March 9 at 3 p.m., animal showed marked weakness. Eye sign present. Symptoms not marked; breathing fairly normal (27 per minute); weight 2055 grams. Bled at 3:30 p.m., venous blood obtained. No urethane given. Cat did not struggle. Survived bleeding 8 to 10 hours, dying between 9 p.m. and midnight.

Cat SC. Maltese, male. Right gland removed March 6; bled from heart, March 9 at 11 a.m. Venous blood. Cat did not struggle. March 16 at 3:30 p.m. re-operated. Left adrenal removed; weight 2310 grams. March 17 at 9:30 p.m., weight 2185 grams. Cat shows adrenal symptoms. 9:30 p.m., marked weakness, unsteady on feet, appears dehydrated. 12:30 a.m., cat very weak, but can walk; not in coma; breathes fairly normally; bled from heart. Venous blood. Animal died in convulsions at 1 a.m.

Cat SB. Maltese. Left adrenal gland removed February 28; bled from heart under urethane; 600 mgm. per kilo at 1:45 p.m. March 4. Cat did not struggle. Arterial blood obtained. Right adrenal removed March 8, at 11:30 a.m., weight 2688 grams. This animal was in very bad condition, March 8, 4 p.m. Second blood not obtained.

Cat V. Female. Operated December 18, 11 a.m. Right adrenal removed. Left adrenal removed December 19 under urethane; bled (800 mgm. urethane per kilo). 10 a.m., December 20, animal dying; typical adrenal death. Very fast respiration. Animal had to be given artificial respiration while bleeding. Note: Animal was dying and respiration ceased before the blood could be withdrawn; hence artificial respiration.

Cat ZE. Large, black and white, male. Weight 3240 grams. Right adrenal removed January 23 at 11:30 a.m. January 25, cat perfectly normal. Given 1.6 grams of urethane by stomach tube at 10 a.m. Bled from heart. Arterial blood obtained (10 cc. venous). This animal died of pneumonia, January 30. The second adrenal was not removed, nor was the blood test completed.

Cat ZF. Large, gray, male. Right adrenal removed January 23 at 12:30 p.m. Weight, 3032 grams. Bled from heart under urethane, January 25 at 12 o'clock. Venous blood obtained. Animal bled again January 29, without urethane. Cat did not struggle. Arterial blood obtained. Cat developed very sore mouth February 8. Salivates continuously. Refuses food. Fed daily by stomach tube. Left

adrenal removed February 8 at 12 o'clock. Animal under ether 38 minutes. Weight, 2951 grams. This animal developed typical adrenal insufficiency symptoms and died at 8:30 p.m., February 8 (e.g., lived 8½ hours). Weight 2931 grams. Lost 20 grams within 8 hours. *No blood obtained after second operation.*

Cat ZI. Maltese, male, adult. Weight 3900 grams. Right adrenal removed January 31 at 3:30 p.m. February 5, animal normal. Was given urethane (800 mgm. per kilo). Bled from heart at 12:15 p.m. Venous blood obtained. Cat did not struggle but began breathing rapidly after withdrawal of 25 cc. of blood. Animal normal at 8 p.m., February 5. February 8, left adrenal removed at 4 p.m. Weight of cat, 2940 grams. Under ether 32 minutes. February 9, animal salivating badly. Shows weakness in hind legs. Shows typical adrenal eye sign peculiar to cats. Refused food at 9 a.m. At noon, February 9, weight, 2860 grams. Skin dry and wrinkled, animal appears dehydrated. Had lost 80 grams within fourteen or fifteen hours. At 3 p.m., February 9, animal given 50 cc. milk by tube. Weight previous to feeding, 2851 grams,—a loss of 9 grams within three hours at room temperature of 73°. Animal did not urinate during this 3-hour period. At 5 p.m. the animal was bled from heart. Arterial blood obtained. Animal perfectly quiet while being bled. At time of bleeding cat was weak, drowsy, and if forced to move about, staggered uncertainly. Respirations were 37 per minute when bled. (This is fairly rapid, the average being 22 to 26 respirations per minute for cats.) Marked evidences of air hunger *were absent.*

Cat ZK. White and gray, male. Weight 2500 grams. Right adrenal removed February 2, 4 p.m. Bled from heart while under urethane (800 mgm. per kilo), 3:30 p.m. Arterial blood obtained. Animal did not struggle.

Cat died February 9 of pneumonia. Left adrenal not removed nor second blood taken.

SUMMARY

1. Heart-blood obtained from cats which had recovered from unilateral adrenalectomy was compared with that obtained after removal of the second adrenal gland. The blood serum was analyzed for the various acid ions, pH, and total base.

2. Consistent differences following bilateral adrenalectomy were:

a. A fall in pH, carbon-dioxide tension, serum bicarbonate, and total acid; indications of an uncompensated non-volatile acidosis.

b. This seemed to be due to an increase of phosphoric and organic acids.

c. Protein also rose, suggesting inspissation of the serum.

d. The nature of the changes is discussed.

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STUDIES ON THE PHYSIOLOGY OF THE LIVER[1]

II. Effect of Adrenalin upon Blood Sugar Following Ligation of the Hepatic Artery[2]

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Blum (1901) was first to demonstrate that the injection of an extract of the adrenal gland into an animal organism results in a glycosuria. Noel-Paton (1902) and Metzner (1902) showed that the glycosuria following adrenalin is dependent upon an increase of the blood sugar concentration. Following these observations, many studies were conducted to establish the dependence of glycosuria and hyperglycemia upon the glycogen reserve of the liver. Adrenalin was administered to animals in which an attempt had been made to free the tissues of glycogen by starvation. Blum (1902) showed that adrenalin glycosuria could still occur in an animal starved for 17 days, and Herter and Richards (1902) obtained similar results in dogs after 24 days of starvation. These observations have been interpreted by others to mean that either starvation does not deplete the organism of its glycogen or that during starvation there is a conversion of fat to carbohydrate as was suggested by Falta (1908). The first hypothesis has proven to be the correct one. Pflüger (1902) was able to obtain 100 grams of glycogen from the liver of a dog starved for 28 days although the animal had lost 23 per cent of its body weight. He was also unable to rid a dog of his glycogen even after 73 days of starvation (1907). Prausnitz (1892) showed that a 22 kgm. phlorhizinized dog fasting 22 days and excreting 287 grams of sugar still contained 25 grams of glycogen in its tissues. Ringer (1910) was successful in ridding starving dogs of their glycogen stores by phlorhizin and shivering, and found that after adrenalin they no longer eliminated extra sugar in the urine as indicated by the persistence of the same D:N ratio.

In order to prove conclusively that adrenalin glycosuria and hypergly-

[1] A preliminary report of this investigation appeared in the proceedings of the Society for Experimental Biology and Medicine, 1926, xxxiii, 545.

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cemia are entirely dependent upon the mobilization of glycogen from the liver, Velich (1903) removed the livers of frogs and was unable to obtain an adrenalin glycosuria. Similarly, Mann and McGath (1923) failed to produce hyperglycemia with adrenalin after total extirpation of the livers of dogs.

In a previous communication (1926) we showed that exclusion of the arterial supply to the liver by ligation of the hepatic artery and its collat-

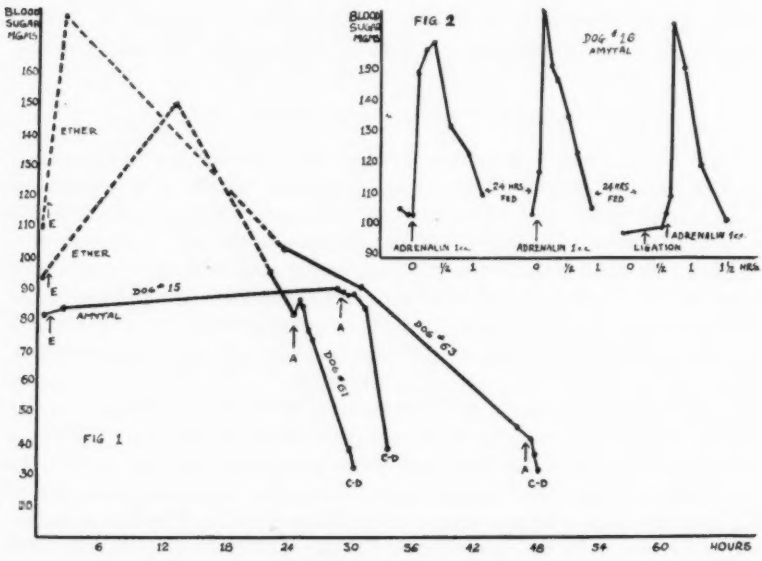

Fig. 1. Effect of adrenalin upon blood sugar concentration at beginning and during hypoglycemic state following exclusion of arterial supply to the liver. *E*, arterial exclusion; *A*, adrenalin intravenously; *C–D*, convulsion and death; broken line, ether effect.

Fig. 2. Control effect of adrenalin upon blood sugar in a normal dog and in the same dog twenty minutes following arterial exclusion of liver.

eral branches results in death with hypoglycemic convulsions. Since it has been previously demonstrated that the development of hyperglycemia and glycosuria after adrenalin injection depends upon the glycogen present in the tissues, it would follow that if the hypoglycemia observed in our animals is associated with a total depletion of glycogen, the injections of adrenalin intravenously during the hypoglycemic state should not elicit a rise in the blood sugar.

The operative procedure for the arterial exclusion of the liver and the

methods of blood analysis have been described by us elsewhere (1926). Dogs were used exclusively and ether or amytal was employed as an anesthetic. Adrenalin P. D. (1–1000) was injected intravenously in amounts varying from 0.5 to 1.5 cc. depending upon the size of the animal. Blood sugar estimations were made on samples taken immediately before the intravenous injections of adrenalin and at short intervals thereafter for a period of one hour.

Results. Figure 1 shows that during the hypoglycemic state, the injection of adrenalin intravenously fails to cause an increase in the blood sugar concentration, although the animals exhibit all the pharmacological effects of adrenalin, such as increased heart rate and force, marked acceleration of respiratory rate and nausea.

That the failure of adrenalin to produce a hyperglycemia is not due to exclusion of the arterial supply to the liver, per se, can be seen from figure 2, where adrenalin was injected 20 minutes after ligation. The blood sugar rise in this case was similar to that which followed the injections done on two previous days, i.e., before ligation. These results are typical of 17 similar experiments. In all cases where the animals died with hypoglycemic convulsions, the liver, heart and skeletal muscles were found by actual analysis to be free of glycogen.

Discussion. The observation that adrenalin fails to produce an elevation of the blood sugar level in animals rendered hypoglycemic by ligation of the hepatic arteries confirms our original observation that exclusion of the arterial supply of the liver causes a total depletion of the glycogen stores of the body. This agrees with the observations of Falta and Priestley (1911) who excluded the liver from the circulation by tying off the vena cava and aorta at the diaphragm and found that when the blood sugar level declined adrenalin failed to increase it. It is also in agreement with Ringer's experiments, in which he was unable to obtain an increase in the excretion of extra sugar by adrenalin from dogs depleted of their glycogen by phlorhizin and shivering. Ohara (1925) was unable to increase the blood sugar level of rabbits poisoned with phosphorus by adrenalin when their blood sugar fell to 60 mgm. per 100 cc. but a rise in blood sugar resulted when he injected adrenalin five minutes after total hepatectomy. This was shown by him to be due to withdrawal of residual glycogen from the muscles.

It seems that the blood sugar level does not necessarily indicate the quantity of glycogen remaining in the organism. Thus in dogs 15 and 61 (fig. 1) with the beginning symptoms of hypoglycemia, 1 cc. of adrenalin injected intravenously had no effect upon the blood sugar although the former contained 82 mgm. and the latter 92 mgm. of sugar per 100 cc. of blood. Five hours later the animals died in convulsions with blood sugars of 35 and 38 mgm. respectively. Similar results were obtained with two

other animals. This is important from a clinical standpoint, for it would seem to indicate that the sugar concentration of the blood is not necessarily an index of the amount of glycogen present in the tissues. Langdon-Brown (1924) reported that symptoms of insulin hypoglycemia may develop in diabetics at a blood sugar level of 150 mgm. per cent; and Leyton (1924) states that insulin shock may occur at a blood sugar level of 250 mgm. per cent when the hyperglycemia of a chronic diabetic is suddenly reduced. On the other hand, cases have been described by Banting (1923) where symptoms of hypoglycemia did not occur at a blood sugar concentration of 32 mgm. per 100 cc. The clinical application of these observations is apparent. It seems, therefore, that the first symptoms of hypoglycemia depend not so much upon the blood sugar concentration as upon the exhaustion of tissue glycogen, and hence adrenalin without glucose would be of no value in increasing the blood sugar concentration during insulin overdosage in diabetics with an abnormally low glycogen reserve.

CONCLUSIONS

1. Adrenalin does not influence the blood sugar level when administered during the hypoglycemic state resulting from exclusion of the arterial supply of the liver.

2. The blood sugar does not necessarily indicate the amount of glycogen present in the organism, since animals on the verge of hypoglycemia but still maintaining a normal blood sugar level may fail to respond to adrenalin with an increased blood sugar level. The failure is not due to a disturbance produced by ligation, but rather to the absence of glycogen from the tissues.

3. The clinical application of these observations has been discussed.

We wish to thank the American Medical Association for a grant which partially defrayed the expense of this investigation. We are also indebted to Miss Elsa Orent and Mr. I. F. Gittlemen for their technical assistance.

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STUDIES ON INORGANIC SALT METABOLISM IN DOGS[1]

III. On Certain Factors which Influence the Deposition and Resorption of Bone

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This report is a brief statement of the general findings obtained during the course of a study on inorganic salt metabolism which has been in progress continuously for the past five years. Some of the conclusions arrived at independently have been reported by workers elsewhere during the course of the study. The method of attack, however, differs from that of most investigators in that we have chosen conditions of diet and hygiene which simulate those encountered in everyday human life. The basal diet, while certainly not optimal, is adequate for rapid growth in normal puppies. No condition which could be attributed to a known dietary deficiency has ever been observed. During the course of the study more than 100 puppies have been studied more or less intensively. The initial skeletal condition of the puppies (with but few exceptions) has been determined by clinical examinations, roentgenographs and blood studies. In many cases observations on the urine were made during a period of 3 to 5 days, and in quite a few, the retention of calcium and phosphorus determined. Observations have been repeated at intervals, as well as before and after a change in the diet. Histological sections and chemical analyses of the bones have been made as a routine, also sections of various organs in certain animals. A detailed report of the findings is in course of preparation and will be published elsewhere in the near future.

The basal diet in the majority of cases has consisted of certain fixed quantities of milk powder, lean beef, butter fat and orange juice supplemented with a sufficient quantity of bread to meet the caloric requirements of the animal. The food was prepared quantitatively, each in-

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* Our experiment reported in this series was conducted in the Department of Household Science, University of California, Berkeley, with the collaboration of Miss Laura James.

gredient being accurately weighed and the whole made into a mush so as to prevent the exercise of choice on the part of the animal. Indoor pens—individual metal cages for the most part—in well-lighted rooms, were used in all of the experiments. The puppies of each litter were divided into 2 or 3 groups—depending upon the number of individuals. The majority of experiments consisted of 3 groups of animals—one group on the basal diet; the second, on the basal diet with a phosphorus free salt mixture added; and the third, on the basal diet and salt mixture supplemented with dibasic potassium phosphate. Different amounts of salt mixture and phosphate were used in the various experiments. The composition of the salt mixture was based on ash analyses of protein free milk made according to the directions of Osborne and Mendel (1), except that the phosphoric acid was omitted, an equivalent amount of hydrochloric and sulphuric acids (proportioned as they occurred in the mixture) being substituted. The potential alkalinity of the salt mixture was varied by adding sodium carbonate or omitting some of the hydrochloric acid.

Basal diet. Thirteen of 27 puppies fed from the time of weaning to 70 days of age or more on a diet consisting of bread, milk, meat, butter fat and orange juice showed no evidence of faulty mineral metabolism. Nine developed rickets-like bone changes to a marked degree (plate 1) and 5 showed more or less evidence of faulty ossification but recovered spontaneously. The addition of $\frac{1}{3}$ to 1.0 gram of sodium carbonate to the diet of three animals after they had passed the age of 77 days had no apparent effect upon calcification. An increase in soda, however, changed active calcification into a retrograde phase in all cases. Hydrochloric acid fed to 2 rachitic animals on the basal diet alone initiated healing (plates 2a–2b).

All of the normal puppies were born during the summer or spring months as were all of the borderline cases. The rachitic were born during the summer and fall. The calcium-phosphorus ratio of the diet during this period was approximately 1 to 1, the absolute amounts of these elements furnished by the average quantity of food ingested being 0.40 gram per dog per day. The protein carbohydrate ratio was 1 to 4; that of fat-carbohydrate 1 to 6.

In no instance has the development of retrograde bone changes been observed in a puppy on the basal diet after he has attained the age of 10 weeks. Some of the animals have been maintained on the basal diet throughout the entire experimental period of several months; others, however, have been subjected to various procedures after they had passed the age at which skeletal changes were likely to occur under this set of experimental conditions.

High calcium diet. The basal diet was supplemented with different amounts of salt mixtures which were relatively high in calcium but varied

in potential alkalinity. In some instances the calcium content of the diet was four times that of the phosphorus.

Twenty-seven puppies were used in this study, only 19 of which were graded as normal at the beginning of the experiment. One hundred per cent of the 11 puppies—10 normal and 1 borderline case—fed on the basal diet supplemented with salt mixtures which were high in calcium but low in potential alkalinity showed satisfactory skeletal development, as far as could be determined by clinical examination, roentgenographs and blood analyses. Six of these puppies were subsequently (at 98 days of age) placed on an alkaline salt mixture, and during a period of 6 weeks, rickets-like skeletal changes developed and progressed rapidly. The 8 borderline cases and 8 normal animals which were placed on salt mixtures containing a larger proportion of basic ions all developed skeletal defects to a greater or lesser degree. Two of the 8 animals which appeared to be normal at the beginning of the experiment and 1 borderline case recovered spontaneously. Two of the normal animals in which rickets like changes had been induced on an alkaline diet containing 4 times as much calcium as phosphorus recovered within 30 days after the neutralization of the excess of alkali with HCl. The control animals showed progressive bone destruction (plate 3).

The addition of the alkaline salt mixture to the diet of animals which give evidence of an inherent metabolic fault has invariably resulted in rapid and profound bone destruction which responds slowly to acid therapy the rapidity of reaction depending upon the degree of metabolic fault. Of the 3 markedly rachitic animals placed on acid therapy, all showed definite evidence of an increase in calcification within 3 weeks, which progressed throughout the experiment. The control animals showed continued bone destruction.

High phosphorus diets. The phosphorus content of the diets as described above was increased by adding various amounts of dibasic potassium phosphate to the salt mixtures used. Twelve animals representing 4 litters were fed on the high phosphorus diet as described. Of these, 10 appeared to be normal at the beginning of the experiment, and 2 showed evidence of faulty mineral metabolism. Seven of the 10 normal animals were placed on a neutral salt mixture. They were litter mates of the 10 normal animals reported in the preceding experiment, and like those animals, failed to develop skeletal defects. in spite of the fact that the phosphorus content of the diet was more than 3 times as great as that of the other group. A subsequent increase in the alkalinity of the diet of three of these puppies resulted, as in the other group, in a reversal of the calcifying mechanism, with the development of marked clinical and roentgenographic evidence of rickets-like changes during a period of 6 weeks. The remaining 3 of the normal puppies were placed on an alkaline salt mixture

and only 1 developed rickets-like bone changes within the usual time (6 to 8 weeks). The addition of ½ to 1.0 gram of soda to the diet of the other 2 animals exerted no appreciable effect upon calcification, the retention of calcium and phosphorus, or the alkalinity of the urine. A further increase in soda, however, caused a marked reduction in the retention of calcium and phosphorus, with roentgenographic evidence of retrograde bone changes. As in the other group of animals, the addition of the high phosphorus salt mixture to the diet of 2 individuals with an inherent metabolic fault resulted in rapid and profound retrograde bone changes. One of these animals was placed on acid therapy. The healing process was initiated within 2 weeks and continued throughout the experiment.

Metabolism studies on various groups of individuals on the basal, high and low phosphorus diets have shown that the reaction of the urine of a puppy with a rachitic tendency is, as a rule, much more alkaline than that of a normal individual on the same diet. Contrary to the findings of Zucker and Matzner (2) on rachitic rats, we have been unable to establish any relationship between fecal reaction and calcification in the few animals studied in this connection. In certain experiments in which the animal was sacrificed at the height of digestion the entire gut was removed immediately, divided into sections and the contents tested. The reaction of the freshly passed feces has in these cases given no indication even of that of the colon.

No striking differences at the time of weaning have been observed in the blood findings of normal and rachitic puppies. In fact, the calcium phosphorus concentration product of the blood plasma of 5 to 7 weeks old puppies which show marked clinical as well as roentgenographic evidence of faulty ossification is usually found to be within the normal range or even high. These findings are in agreement with the recent report of Wilson (3) in a study of rachitic infants. As the rachitic process progresses the calcium-phosphorus concentration product of the plasma almost invariably decreases. During the early stages of a retrograde phase of calcification, the blood phosphorus of an older animal on a high phosphorus diet tends to be higher, and the blood calcium lower than that of the control animal on the low phosphorus diet. Likewise, an excess of one element in the diet causes an increase in the retention of that element and a reduction in the other, under certain conditions, but not always. These findings are in agreement with those of Orr (4) and others and have been interpretated as indicative of faulty absorption in the intestinal tract due to a precipitation of insoluble calcium phosphate. In groups of normals animals on diets which are relatively low in potential alkalinity, metabolism experiments have shown that the retention of calcium and phosphorus may be of the same order of magnitude regardless whether the phosphorus content is high or low, within certain limits (groups 1 and 2, table 1). The

TABLE 1

A summary of metabolism findings on groups of animals of the same litter, on the same food intake

LITTER 1		INGESTED					EXCRETED						RETAINED		PER CENT EXCRETED				PER CENT RETAINED		PER CENT OF TOTAL EXCRETED IN URINE		CALCIFICATION	
				Salt mixture			Urine		Feces		Total				Urine		Feces							
Group	Age	Ca	P	Grams	Alkalinity	Reaction	Ca	P	Ca	P	Ca	P	Ca	P	Ca	P	Ca	P	Ca	P	Ca	P	Beginning	Ending
	days	*gms.*	*gms.*				*gms.*	*gms.*	*gms.*	*gms.*	*gms.*	*gms.*	*gms.*	*gms.*	*gms.*	*gms.*	*gms.*	*gms.*						
1	93	0.80	1.34	3.0	Low	*Neut.	0.110	0.833	0.298	0.188	0.309	1.021	0.490	0.319	1.4	62.2	37.3	14.0	61.3	23.8	3.6	81.6	+	+
2	80	0.80	0.40	3.0	Low	*Neut.	0.031	0.005	0.201	0.088	0.231	0.093	0.570	0.307	3.9	1.3	25.1	21.7	71.1	77.0	13.5	5.4	+	+
1	133	1.2	1.49	6.0	Alk.	Alk.	0.041	0.797	1.122	0.493	1.161	1.289	0.039	0.202	3.4	53.5	93.4	33.0	3.2	13.5	3.5	61.8	+	−
2	133	1.2	0.555	6.0	Alk.	Alk.	0.051	0.116	0.890	0.401	0.941	0.517	0.259	0.038	4.3	21.0	74.1	72.1	21.6	6.9	5.4	22.4	+	−
2	152	0.444	0.485	0		Alk.	0.034	0.332	0.484	0.192	0.518	0.524	−0.074	−0.039	7.7	68.5	109.0	39.6	−16.7	−8.0	6.6	63.4	−	− −
1	170	0.444	1.42	0		Alk.	0.011	1.185	0.867	0.389	0.878	1.574	−0.434	−0.154	2.5	83.5	195.3	27.4	−98.0	−10.9	1.3	75.3	−	− −
2	179	0.444	0.45	0		Alk.	0.087	0.345	0.530	0.210	0.617	0.555	−0.173	−0.070	19.6	71.2	119.4	43.3	−39.0	−14.4	14.1	62.1	−	− −

* Litmus used as indicator.

Explanation of tables and charts. Metabolism periods were from 3 to 5 days each.

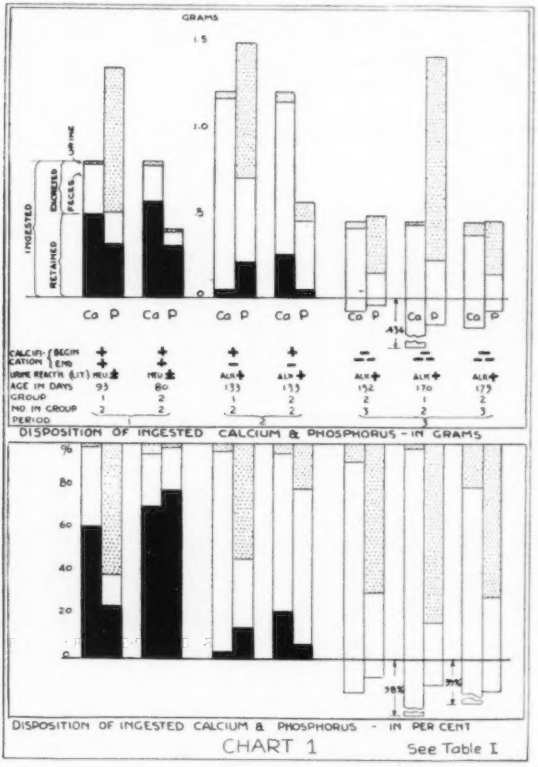
The absolute amounts in grams (ingested, excreted, etc.) represent daily averages for each animal.

The findings on various animals of the same litter on the same food intake were comparable and are given as averages.

Calcification is indicated by + (active) and − (retrograde) and refers to condition at the beginning and ending of the period during which the metabolism study was made.

Charts are graphic representations of data given in tables.

reaction of the urine varied around the neutral point (pH 7.0). Interesting differences occurred in the excretion of phosphorus by two groups of animals (litter mates). Those on the high phosphorus diet excreted a large percent of this element ingested in the urine, only a trace occurring in that of the high calcium group (groups 1 and 2, table 1). Here it seems there must have been a specific interaction of ions. Yet there was no



apparent difference in ossification, and the retention of these elements by the individuals of the two groups was remarkably comparable. The metabolism of two puppies (litter mates) on a high calcium alkaline diet during a retrograde phase of calcification was strikingly different from that of the majority of animals on similar diets. Both of these puppies retained more calcium and phosphorus than the normal controls on the basal diet (groups 1 and 2, table 2). Sixty-six per cent of the total cal-

TABLE 2

A summary of metabolism findings on groups of animals of the same litter, on the same food intake

LITTER 2		INGESTED							EXCRETED						RETAINED		PER CENT EXCRETED				PER CENT RETAINED		PER CENT OF TOTAL EXCRETED IN URINE		CALCIFICATION	
				Salt mixture					Urine		Feces		Total				Urine		Feces							
Group	Age	Ca	P	Grams	Alkalinity	Supplement Na_2CO_3	Supplement $\frac{N}{10}$ HCl	pH	Ca	P	Ca	P	Ca	P	Ca	P	Ca	P	Ca	P	Ca	P	Ca	P	Beginning	Ending
	days	*gms.*	*gms.*	*gms.*		*gms.*	*cc.*		*gms.*	*gms.*	*gms.*	*gms.*	*gms.*	*gms.*	*gms.*	*gms.*										
1	42	0.395	0.383	0		0	0	6.8	0.021	0.132	0.050	0.043	0.071	0.175	0.324	0.208	5.4	34.5	12.6	11.2	82.0	54.3	30.0	75.5	+	+
1	70	0.442	0.447	0		0	0	7.3	0.010	0.194	0.048	0.058	0.058	0.252	0.384	0.195	2.3	43.4	10.8	12.9	87.0	43.7	17.3	77.0	+	±
2	70	1.262	0.454	6	Mod.	0	0	7.8	0.387	0.057	0.200	0.087	0.587	0.143	0.675	0.311	30.7	12.6	15.8	19.1	53.5	68.5	66.0	40.0	+	−
3	70	1.262	1.014	6	Mod.	0	0	7.3	0.016	0.304	0.418	0.205	0.434	0.509	0.828	0.505	1.3	30.0	33.1	20.2	65.6	50.0	3.7	60.0	+	+
1	90	0.46	0.483	0		0.5	0	7.6	0.009	0.130	0.030	0.106	0.039	0.236	0.421	0.247	2.0	27.0	6.5	21.9	91.5	51.2	23.0	55.0	±	+
2	90	1.262	0.454	6	Mod.	0	0	7.9	0.412	0.133	0.171	0.081	0.583	0.214	0.679	0.240	32.7	29.3	13.6	17.8	53.8	53.0	70.7	62.0	−	−
2	90	1.280	0.490	6	Mod.	0	35	7.4	0.493	0.018	0.092	0.070	0.585	0.088	0.695	0.402	38.3	3.7	7.2	14.3	54.3	82.0	84.3	20.0	−	+
3	90	1.280	1.142	6	Mod.	0.5	0	7.4	0.025	0.409	0.426	0.208	0.453	0.615	0.828	0.527	2.0	35.8	33.3	17.3	64.7	46.2	5.5	65.5	+	+
3	110	1.280	1.437	6	Mod.	0	0	7.2	0.008	0.670	0.494	0.254	0.502	0.924	0.778	0.513	0.6	46.7	38.6	17.6	61.0	35.7	1.6	72.6	+	+
1	173	0.498	0.566	0		1.5	0	7.8	0.030	0.214	0.152	0.105	0.182	0.319	0.316	0.247	6.0	37.8	30.5	18.5	63.5	43.7	10.4	67.0	+	−
3	173	1.318	1.518	6	Mod.	3.0	0	7.7	0.024	0.783	0.881	0.431	0.905	1.214	0.413	0.304	1.8	51.6	66.8	28.4	31.3	20.0	2.7	64.5	+	−

cium and 40 per cent of the total phosphorus excreted was found in the urine. Metabolism studies repeated three times at intervals revealed the same peculiarity, yet rickets-like skeletal changes progressed rapidly. From this it appears that the absolute amounts of calcium and phosphorus retained as well as the absolute amounts of these elements ingested, is not the factor which controls the deposition of bone salts. An argument in support of this view is the beneficial effect of inanition on calcification. Since the products of katabolism of body tissue contain a large excess of phosphorus over calcium, it becomes apparent that neither concentration nor proportion of these elements are of paramount importance in the deposition of bone in such cases.

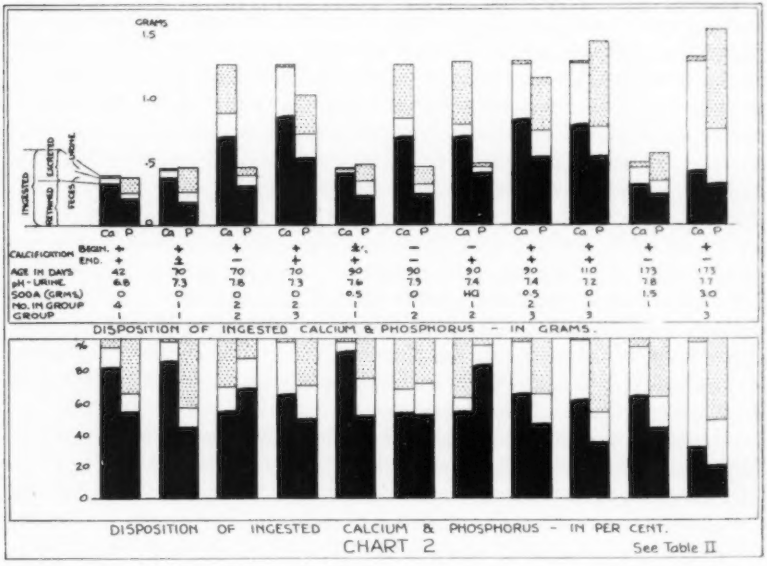


CHART 2 See Table II

An increase in the alkalinity of the diet in certain instances caused a tremendous increase in fecal calcium and phosphorus, regardless whether the phosphorus content of the diet was high or low. In some cases there has been a concomitant increase in both elements in the urine; (group 2, table 1) in others, no appreciable effect was observed. The fact that so large a percentage of the ingested phosphorus, and in two instances as much as 30 per cent of the ingested calcium, was excreted in the urine, contraindicates faulty intestinal absorption. A study of the metabolic, roentgenographic and blood findings on a large number of animals under controlled conditions leads us to believe that an increase in the fecal cal-

cium and phosphorus in human and experimental rickets is the result of the excretion of these elements into the intestinal tract, and is due to the inability of the bone-forming cells to utilize the material which may be furnished them in abundance. This is contrary to the generally accepted view, which holds that the seat of the disturbance in human and experimental rickets is primarily in the gastro-intestinal tract. Further evidence in support of the view that a failure of calcification is due primarily to faulty utilization by the tissues, rather than faulty intestinal absorption of calcium and phosphorus, is to be found in the large negative balances of these elements which occurred in certain animals, already undergoing retrograde bone changes, upon the sudden withdrawal of the alkaline salt mixtures (period 3, table 1). The urine of all the animals (five) remained highly alkaline and contained a large percent of the excreted calcium and phosphorus, which would contraindicate faulty absorption. Furthermore, it is difficult to conceive how negative balances could occur on satisfactory diets unless there was faulty utilization of these elements by the tissues. Simple deprivation of food does not of itself produce skeletal defects; in fact, it is generally conceded that inanition promotes calcification in both rachitic infants and experimental animals. On diets containing an excess of calcium or phosphorus with an optimal reaction in the intestinal tract, the retention of these elements by the animals in the two groups (high calcium and high phosphorus) was remarkably comparable. When the reaction was unfavorable—that is, too alkaline—a high concentration of one element apparently caused a precipitation of both elements in the form of insoluble calcium phosphate, a relative excess of the abundant element being absorbed, although in absolute amounts, the retention of both elements was reduced.

Another factor which seems to have an important bearing on the metabolism of calcium and phosphorus is the total salt concentration. In certain groups of animals in which rickets-like bone changes had been induced on alkaline diets both high and low in phosphorus, the sudden withdrawal of the phosphorus free salt mixture greatly accelerated the rachitic process. An astonishing amount of bone destruction occurred in a period of 12 days in all of the 9 animals on both the high and low phosphorus diets. The addition of dibasic potassium phosphate to the diet of 2 of these animals further accelerated bone destruction—so much so that the ash content of their bones at 265 days of age was approximately one-half of that of normal newborn puppies. Direct exposure to the sun for several hours a day for a period of 3½ months failed to promote calcification in 3 of these animals. Here it seems that the calcifying mechanism was permanently injured by the sudden alteration in the salt concentration of the tissue fluids. This phase of the problem is being further investigated.

Finally, the reversal of the calcifying mechanism in one leg of an ani-

mal undergoing retrograde bone changes on a decalcifying diet by frequent exposure to x-rays, localized the seat of the disturbance in faulty mineral metabolism in the bone cell itself (plate 4).

Faulty calcification is visualized as follows:

A certain excess of alkali in an otherwise satisfactory diet results in the precipitation of some of the calcium salts in the intestinal tract and their ultimate excretion. The easily soluble alkali has a twofold effect—reduction in the available calcium salts in the intestinal tract and an increase in alkalinity of the tissue fluids. Howland (5) has shown that a difference of 0.02 in pH in the body fluid is sufficient to determine the site of precipitation of calcium salts. With a slight increase in alkalinity the calcium-phosphorus concentration product of the tissue fluids would be reduced, which in turn would cause virtual starvation of the bone cells and a corresponding decrease in oxidation. Stieglitz (6) has shown the effect of membrane on the hydrolysis of salts such as a sodium salt with a non-diffusible anion. Applying Donnan's law he found that the more acid a solution is on one side of a membrane the more alkaline it must be on the other side. If this is true, and the intercellular fluid is more alkaline than normal, we should expect the intracellular fluid to be more acid. Under this condition there would be a failure of calcification. With a reduction in oxidation one would expect a reduction in CO_2 tension and an increase in pH. This is perhaps what occurs in the early stages, but if the CO_2 tension is further reduced it seems reasonable to assume that there would be an increase in organic acids which would ultimately result in destruction of the bone cell and solution of bone salts. This, perhaps, is what occurs in so-called halisteresis in which there appears to be a solution of bone without the intervention of cells. If, on the other hand, the activity of the bone-forming cells is only slightly impaired, the accumulation of acid might be less and function suspended rather than destroyed. At this point calcification could be initiated in two ways: first, the correction of the reaction of the tissue fluids by dietary means, which would ultimately result in the restoration of a normal calcium-phosphorus concentration product. This is what actually happens when the excess of alkali to the diet is neutralized with hydrochloric acid. Concomitant with this there would be better absorption of calcium and phosphorus from the intestinal tract. Restoration of a more favorable environment for the cell with an adequate food supply would, if activity were only suspended, ultimately restore its function, and calcification would be resumed. Obviously this procedure would be slow in cases of marked hypocellular function. The second method of promoting calcification would be the direct application of any agent which would sufficiently stimulate the cell to enable it to overcome the effects of its unfavorable environment. With an increase in cell activity there would be an increase in oxidation, an increase in CO_2 and

a decrease in organic acid—the final result being the restoration of an optimal balance of ions and the resumption of calcification.

In spontaneous rickets, both human and experimental, occurring on satisfactory diets, the sequence of events is probably just the reverse of that described. Here it seems that the bone cells are unable to handle the material which, in early stages at least, is furnished them in abundance. Faulty calcification, within certain limits, would be a failure of deposition rather than decalcification, and any external factor such as diet, hygiene or ultraviolet radiations would play an important part for or against the rachitic process.

According to Stieglitz, as the alkalinity within the cell increases, that without decreases. The calcium-phosphorus concentration product of the plasma would return to normal. The optimal balance between cell and tissue fluids would be restored and calcification resumed. If this is true, any agent which, directly or by catalysis, will stimulate the activity of bone cells, will promote calcification. Such is believed to be the action of cod liver oil and ultra violet light. This places faulty mineral metabolism in the same category of diseases as cretinism and diabetes, both of which are the result of hypocellular function due to the absence of specific stimuli.

SUMMARY

Normal puppies kept in indoor pens grew satisfactorily and showed no evidence of faulty mineral metabolism on diets consisting of certain fixed quantities of meat, milk, butter fat and orange juice supplemented with a sufficient quantity of wheat bread to meet the caloric requirements of the growing animal. Puppies with an inherent metabolic fault developed more or less marked rickets-like bone changes on the same diet under identical conditions.

The retention of calcium and phosphorus by normal puppies on the basal diet with the addition of a phosphorus-free salt mixture high in calcium and low in potential alkalinity; and the same, supplemented with sufficient dibasic potassium phosphate to make the phosphorus content relatively high, were of the same order of magnitude. Skeletal development appeared to be normal in all cases. Subnormal puppies showed profound retrograde bone changes on the same diets and under identical conditions.

Normal animals tolerate well a certain excess of alkali in diets both high and low in phosphorus, but if the amount is sufficiently increased, the calcifying mechanism is reversed. Neutralization of the excess of alkali initiates the healing process, which progresses rapidly in animals in which retrograde bone changes have been induced and are not too advanced, and more slowly in those animals with an inherent metabolic fault.

The calcium-phosphorus concentration product of the blood plasma was found to be well within the normal range in young puppies in the early stages of rickets. In more advanced cases in older animals the phosphorus content was markedly reduced.

Metabolism and blood studies indicate that the seat of the disturbance in faulty mineral metabolism is in the tissues rather than the gastrointestinal tract.

It appears that calcification is dependent upon two factors—one, and the more important, being the metabolic state which is inherent in the individual, and the other—diet, which plays an important part in regulating cell metabolism. It is believed that the precipitation of bone salts is determined by the balance of ions between the bone forming cells and their surrounding medium, and that any agent (diet, hygiene, ultraviolet light, active principle in cod liver oil, etc.) which either directly or by catalysis influences the metabolism of the bone cells will alter that balance and thus become an important factor in regulating the deposition and resorption of bone.

A theory of the mechanism of calcification is given.

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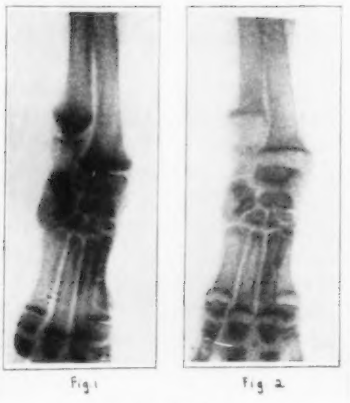

PLATE I

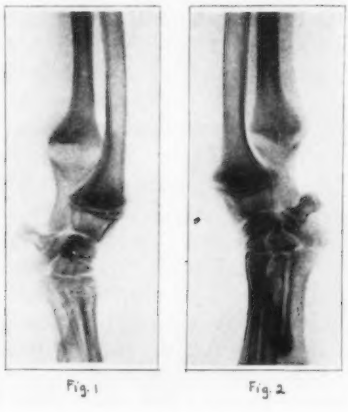

PLATE IV

Plate I: Roentgenographs of the left fore legs of full brothers of the same age but of different litters. Both were fed on the basal diet exclusively.

Fig. 1. Age 77 days. Normal skeletal development.

Fig. 2. Age 77 days. Rapid and profound retrograde bone changes.

Plates II a and II b: Fig. 1. Age 70 days. Spontaneous rickets on basal diet.

Fig. 2. Age 128 days. Basal diet and cod liver oil from 91st to 130th day. Note increase in calcification at epiphyseal ends of ulna and radius.

Fig. 3. Age 145 days. Basal diet and 1.0 to 1.5 grams of sodium carbonate from 130th to 145th day. Continued healing.

Fig. 4. Age 188 days. Basal diet and 1.5 to 3.0 grams of sodium carbonate from 145th to 188th day. Retrograde bone changes. Note resorption of metaphysis of ulna.

Fig. 5. Age 270 days. Basal diet and 3.0 to 4.5 grams of sodium carbonate from 188th to 270th day. Calcification static. Dog refused food. Evidence of healing during the last two weeks.

Fig. 6. Age 287 days. Sodium carbonate omitted from diet and 20 cc. of normal hydrochloric acid added from the 270th to 287th day. Rapid healing.

Plate III: Roentgenograms of the left fore legs of two puppies of the same litter on the basal diet supplemented with a salt mixture high in both calcium and potential alkalinity. The calcium content of the diet was four times that of the phosphorus.

Figs. 1 and 2. Puppies Nos. 1 and 2 respectively. Age 100 days. Note widening epiphyseal zones at "a" and "b" in both animals.

Fig. 3. Puppy No. 1. Age 130 days. No change in diet. Note progressive bone destruction at "a" and "b".

Fig. 4. Puppy No. 2. Age 130 days. Excess of alkali in diet was neutralized with hydrochloric acid. Note increase in calcification at "a" and "b".

Plate IV: Roentgenographs of the right and left fore legs of the same animal on the 128th day of age. Retrograde bone changes induced on the basal diet supplemented with a salt mixture high in both calcium and potential alkalinity. The left fore leg was exposed to x-rays at frequent intervals.

Fig. 1. Right fore leg. Note wide zone of uncalcified matrix at epiphyseal end of ulna. Rachitic process active.

Fig. 2. Left fore leg. Note increase in calcification at epiphyseal end of ulna. Healing initiated locally by x-rays in spite of an unfavorable diet.

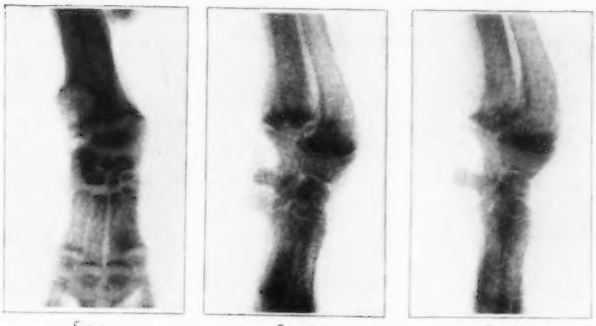

Fig 1 Fig 2 Fig 3

Plate II-a

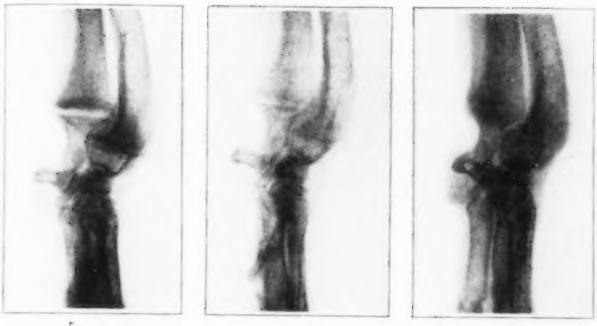

Fig 4 Fig 5 Fig 6

Plate II-b

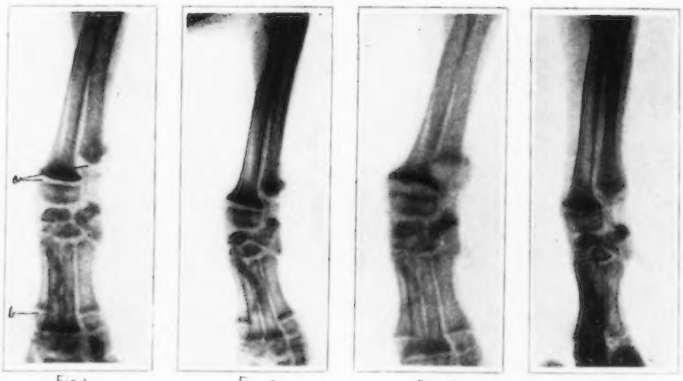

Fig 1 Fig 2 Fig 3 Fig 4

PLATE III

THE EFFECT OF THE BLOOD CALCIUM LEVEL ON THE TOLERANCE TO MAGNESIUM

Some Observations on Hypercalcemia Induced by the Parathyroid Hormone

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The organism exists and does its work in an environment to which it is peculiarly adapted. In the animal, as a whole, the active tissues are bathed by a fluid medium of relatively constant composition. Any change in the constituents of this physiological medium is reflected by changes in the activity of the tissues. Aside from the physical forces, osmotic in nature, exerted by the fluid medium, the effects upon the activities of tissues induced by a variation of the inorganic constituents of the fluid medium have been the subject of many investigations. Of the inorganic salts usually found in the physiological fluid medium none have attracted greater attention than those of calcium and magnesium, which are dealt with in this study.

Magnesium is poisonous to all forms of life, exerting a general depressant effect. On contractile tissues, e.g., muscle, it depresses the irritability and lessens the tone. Loeb (1902) showed that magnesium salts increased the threshold of excitability and allayed the muscular twitchings caused by changes in the concentrations of certain salts in solutions bathing the muscle. This magnesium effect is forcibly demonstrated in stopping the muscular twitchings caused by a reduction of the calcium content of the environmental medium, and the muscular twitchings, tetany, caused by a reduction in the calcium content of the blood plasma.

Meltzer and Auer (1908) were the first to subject magnesium to a critical pharmacological investigation. Working with mammals (rabbits and dogs) they showed its action to be that of a general depressant. As with other depressants, such as anesthetics, the first tissue to succumb was that of the nervous system, resulting in a state of general anesthesia. It was also shown that magnesium salts blocked the nerve impulse when applied locally to the nerve trunk or injected into the subdural space, and thus led to a condition of local anesthesia. Nerve endings (motor and sensory), nerve centers, synapses, neuro-muscular connections, nerve

trunk, in fact any point in the reflex arc is subject to this blocking effect of depression by magnesium.

In mammals the immediate cause of death from magnesium administration seems to be due to the failure of the respiratory center. Secondary to this effect of magnesium, a depression of the vasomotor apparatus becomes apparent, as well as a depression of the neuro-muscular mechanism for the propagation of the heart beat. The heart is slowed and dilated, and tends to stop in diastole. The blood vessels become relatively dilated. As a result of the circulatory effects of magnesium there is a considerable drop in blood pressure, as was shown by Matthews and Brooks (1910).

Calcium salts act upon skeletal muscles with an effect resembling that of magnesium, but with far less intensity. On isolated muscle preparations, such as the gastrocnemius of the frog, the withdrawal of calcium from the surrounding or permeating fluid increases the irritability and leads to spontaneous muscular twitchings which resemble tetany (Loeb, 1902). The addition of calcium salts to the calcium impoverished solution brings the tetany to a stop and even renders the muscle less irritable. In the intact animal a reduction of the calcium content of the blood plasma by the injection of sodium oxalate, or following parathyroidectomy, leads to a condition of muscular twitchings called tetany. This tetany can be quickly relieved by the injection of soluble calcium salts, or by the administration of an extract of the parathyroid glands, as outlined by Collip (1926). The point of action of the calcium on the isolated muscle preparations is on the muscle itself, but some have suggested that calcium salts act also upon the myoneural junction, like curare.

On all musculature which is involuntary, and which possesses a rhythmical contractility, including the heart and blood vessels, calcium exerts a different effect. This is especially seen in studies of the heart and vascular system. Kylin and Nystrom (1925) have observed an adrenalin-like effect on blood pressure of man following injection of calcium salts. Calcium salts increase the strength of the heart beat and the degree of contraction (systole) and decrease the degree of relaxation (diastole), thus making the organ smaller, and finally bring the heart to a standstill in systole. This action resembles that of digitalis to such a marked degree that clinicians at various times tried to substitute calcium salts for digitalis. The poisonous effect of large doses of calcium seems to be due mainly to the slowing and incomplete filling of the heart. Hamburger (1922) has observed that calcium salts produce marked contraction of the vessels.

The mutual antagonism of calcium and magnesium was first described by Meltzer and Auer (1908), who were able to relieve immediately the general magnesium depression by the injection of soluble calcium salts.

Subsequent workers have sought to explain the mechanism of the antagonism in various ways, viz.: ionic displacement of magnesium by calcium, colloidal changes of hydration and dehydration due to the alteration of the calcium and magnesium content of the fluid, complex ion formation, etc. The recent work of Baumecker (1923) is prefaced by a good review of the various theories and the experimental work underlying them. Mendel and Benedict (1909) have shown that injection of calcium salts results in an increase in the elimination of magnesium, and that the injection of magnesium salts causes an increase in the elimination of calcium. Richter-Quittner (1925) has shown that oral and intravenous administration of magnesium chloride renders the calcium of the blood more diffusible. While no one explanation of the mechanism of the antagonism seems adequate, it is generally accepted that the concentrations of sodium, potassium, calcium and magnesium salts must be kept relatively constant for the maintenance of normal conditions and processes.

The object of our experiments was to determine the effect of magnesium administration to animals with abnormal concentrations of blood calcium.

Several methods of producing changes in the calcium concentration of the blood are now available. MacCallum and Voegtlin (1909) showed that the removal of the parathyroid glands of the dog results in a state of prolonged hypocalcemia, the calcium level falling from the normal of 10 to 11 mgm. per 100 cc. of serum to values of 5 to 7. Gross (1923) has shown that the subcutaneous injection of oxalates produces a lowering of the blood calcium. Accompanying the fall in the blood calcium the syndrome of tetany was observed. Many subsequent workers have confirmed the findings of MacCallum and Voegtlin. Particularly valuable are the studies of Collip (1926) who has prepared an extract of the parathyroid glands which acts in elevating the blood calcium. By the administration of this parathyroid hormone to normal dogs a prolonged state of hypercalcemia may be induced. We have made use of these three methods to produce changes in the calcium content of the blood. Irving (1926) has also produced extreme hypercalcemia in dogs by introducing a mixture of calcium chloride, acetic acid and sodium acetate into the small intestine, with the pylorus ligated and the animal under general anesthesia.

In a study of this nature we found it advisable to determine on normal animals the effect of injections of magnesium sulphate. Joseph and Meltzer (1909–10) found that 0.223 gram of magnesium chloride per kilo was a fatal dose of the salt for the dog. But their injections were made in several divided doses, into different vessels, cannulated and under local anesthesia.

We chose dogs for the experimental animals and for the injecting solution a 20 per cent solution of the heptahydrated magnesium sulphate

($MgSO_4 \cdot 7H_2O$). Just a few minutes before the injection the blood for calcium analysis was drawn from the leg vein or, in a few instances, from the heart. The blood was drawn upon powdered heparin, which delayed coagulation. The sample was then centrifuged and 2 cc. portions of the plasma used for each analysis by the Tisdall (1923) method. Nearly all of the values given for calcium content represent the averages of duplicates, which agreed within less than 1 mgm. per 100 cc. of plasma. In a few instances the blood calcium determinations were omitted because previous analyses had shown the animal to be normal in this respect. Only a few minutes after bleeding the dog for the calcium analysis the solution of 20 per cent magnesium sulphate was injected. The injection was made in one application into the intact leg vein and at such a rapid rate that the entire injection rarely required over one and one-half minutes. After some of the injections, when it was thought that the dog was in danger of immediate death, the animal was revived by quickly injecting 5 to 15 cc. of 5 per cent calcium lactate. In a few instances the animals had so far succumbed that they did not respond to the calcium lactate.

The results of these studies of the magnesium tolerance of normal animals are given in table 1, and indicate that 0.23 to 0.28 gram of the hydrated magnesium sulphate per kilo is the fatal dose for the dog with a normal blood calcium, although smaller doses often resulted in unconsciousness, gasping, panting and unsteadiness in gait. The response to the injections was immediate but short in duration, lasting five to fifteen minutes. No prolonged anesthesia was observed as a result of the intravenous injection.

Some comment should be made upon the results of the experiments with animals M10, M25, M26, M29. These animals received several injections of the magnesium solution, but on different days, and were either unusually resistant to the magnesium effect from the first or developed a tolerance with succeeding injections. It will be noted that the first injection to each of these animals was always below that found fatal for the larger number of animals. From a consideration of these facts, and the responses of the animals to the several injections, we are inclined to the opinion that the normal dog develops a tolerance for the salt.

In order to determine the tolerance of the hypercalcemic dog to magnesium sulphate, these animals were first rendered hypercalcemic by subcutaneous injections of "Parathormone" (Collip) (Eli Lilly & Co.). The hormone preparation was usually injected on the afternoon of the day before the injection of the magnesium sulphate solution, and the resultant calcium elevation maintained, or increased, by daily injection of the hormone. Bleeding for calcium analyses and injections of the 20 per cent solution of magnesium sulphate were conducted as described above.

All degrees of hypercalcemia, from 11.37 to 24.38 mgm. of calcium per 100 cc. of plasma are represented, with simultaneous injections of varying quantities of magnesium sulphate solution, in table 2.

TABLE 1

Magnesium tolerance by animals with normal blood calcium

DATE	ANIMAL NUMBER	WEIGHT	VOLUME OF 20 PER CENT $MgSO_4 7H_2O$ INJECTED	AMOUNT OF THE SALT PER KILO	Ca IN 100 CC. OF PLASMA	RESULTS OF THE INJECTION
		kgm.	*cc.*	*gram*	*mgm.*	
5-17-26	M13	9 0	8 0	0.180	Normal	Animal nearly died but recovered
4-21-26	M7	9.5	11.0	0.232	Normal	Died instantly
5-10-26	M13	9.0	8.0	0.180	9.58	Revived by artificial respiration
5-14-26	M10	4.0	4.5	0.225	10.64	Panting. Recovering. (5th injection)
5-17-26	M10	4.0	8.0	0.400	Normal	No great distress. Discarded this animal. (6th injection)
7-28-26	M28	11.0	15.0	0.273	10.82	Died immediately
5-12-26	M14	9.0	10.0	0.222	10.86	Prostration. Recovery
4-26-26	M10	4.0	7.0	0.280	11.37	Heart accelerated
7-27-26	M25	12.0	15.0	0.250	11.22	Gasping. Recovery. (4th injection)
8-10-26	M33	11.0	15.5	0.281	11.42	Died before revival with calcium lactate could be made
7-29-26	M29	11.0	15.0	0.273	11.51	Prostration. Recovery. (1st injection)
7-30-26	M29	11.0	20.0	0.364	11.51	Gasping. Recovery. (2nd injection)
8- 2-26	M30	15.4	23.0	0.300	11.61	Revived with 10 cc. of 5 per cent calcium lactate intravenous
7-23-26	M26	10.0	11.0	0.220	11.61	Unconsciousness. Panting. Recovery. (1st injection)
8-10-26	M31	10.5	15.0	0.285	11.82	Revived with 15 cc. of calcium lactate intravenous
7-23-26	M25	12.0	12.5	0.210	12.21	Prostration. Recovery. (2nd injection)
8-10-26	M32	10.5	15.0	0.285	12.52	Died before calcium lactate could be given
7-23-26	M27	21.4	34.0	0.317	12.70	Died instantly
7-22-26	M25	12.0	11.0	0.183	12.80	Unsteadiness. Recovery. (1st injection)
7-25-26	M25	12.0	15.0	0.250	Normal	Recovery. (3rd injection
7-28-26	M25	12.0	17.0	0.283	Normal	Recovery (5th injection)
7-30-26	M25	12.0	20.0	0.333	Normal	Recovery. (6th injection)
7-27-26	M26	10.0	20.0	0.400	Normal	Recovery. (3rd injection)
7-28-26	M26	10.0	22 0	0 440	Normal	Died immediately. (4th injection)
7-27-26	M26	10.0	12.0	0.240	Normal	Recovery. (2nd injection)

TABLE 2

Magnesium tolerance by animals with elevated blood calcium

DATE	ANIMAL NUMBER	WEIGHT	VOLUME OF 20 PER CENT $MgSO_4 7H_2O$ INJECTED	AMOUNT OF THE SALT PER KILO	Ca IN 100 CC. OF PLASMA	RESULTS OF THE INJECTION
		kgm.	*cc.*	*gram*	*mgm.*	
4-26-26	M10	4.0	7.0	0.350	11.37	Heart accelerated. (1st injection)
5-17-26	M16	11.5	20.0	0.348	11.70	Died
5- 8-26	M10	4.0	5.5	0.252	12.83	Very little effect. (4th injection)
4-28-26	M10	4.0	8.0	0.400	13.00	Nearly died but quickly recovered. (2nd injection)
5- 5-26	M10	4.0	5.0	0.250	13.16	Very little effect. (3rd injection)
4- 6-26	M2	11.0	22.0	0.400	13.92	Great distress. Revived with injection of calcium lactate
5-12-26	M16	11.5	16.0	0.287	13.93	Some gasping. Recovery in short time
8- 4-26	M30	15.4	24.0	0.312	14.09	Recovery. Heart rate changed from 72 to 120
3-31-26	M1	11.0	20.0	0.364	14.10	Unsteady in gait for a few minutes. Recovery
4-14-26	M4	8.2	12.0	0.293	14.40	Little effect except to change heart rate from 50 to 120
4-10-26	M3	7.0	20.0	0.571	14.41	Died. Heart had been injured by previous puncture
4-23-26	M8	7.5	9.0	0.240	16.80	Heart relaxed and accelerated. Recovery
4-23-26	M9	5.5	6.0	0.220	17.00	Heart relaxed. Recovery
5- 1-26	M13	9.0	11.0	0.247	17.29	Little effect. Recovery
4-16-26	M4	8.2	10.0	0.244	18.35	Recovery. Animal seemed better
5- 1-26	M14	9.0	10.5	0.233	19.93	Little effect. Recovery
4-15-26	M5	6.0	12.0	0.400	23.30	Died. Calcium on vena cava blood post mortem
4-15-26	M4	8.2	9.0	0.220	24.38	Heart rate increased from 50 to 120. Recovery

Our results show that the tolerance of the dog to magnesium sulphate solution is definitely increased when the blood calcium is increased. The fatal dose of 0.28 gram per kilo for normal animals was in no case fatal when introduced into the circulation of the hypercalcemic animal. In fact, from 0.30 to 0.32 gram of the salt per kilo was very well tolerated, while amounts over 0.35 gram per kilo were usually fatal. In addition to showing an increased tolerance to magnesium sulphate solution, we observed non-fatal doses of the salt did not produce nearly as much depression and collapse of the hypercalcemic as of the normal animal. Usually these animals with elevated blood calcium were able to walk with only slight unsteadiness in two to five minutes after the injection.

Another point of antagonism of calcium by magnesium was observed in studies of the heart, during and before hypercalcemia and the injections of magnesium sulphate. Coincident with the hypercalcemia produced by the hormone the heart became so contracted as to make bleeding by heart-puncture a matter of difficulty. This contraction of the heart was always accompanied by irregularities in the beat or actual slowing of the heart, or both. Immediately after the injection of magnesium sulphate solution the heart dilated to normal size and returned to its normal rate and rhythm. This magnesium effect lasted only one-half to two hours, after which the heart returned to its original hypercalcemic condition.

This marked contraction and irregularity of the heart accompanying hypercalcemia, and antagonized by injections of magnesium sulphate, has been observed also directly by us, by opening the thorax of the animal under ether anesthesia, with artificial respiration. We have also noted, in autopsy on animals dying after overdosage of the hormone, that the heart is small, hard, and contracted to such an extent as to contain very little blood.

The effect of the hormone and the magnesium upon the heart are clearly shown by the following protocol, abbreviated from our record of dog M4:

Dog M4. Male. Weight 8.2 kilos.

4/13/26. At 10:40 a.m. the blood calcium was 9.40 and the heart rate 120. One hundred units of the parathormone were injected at 11 a.m. and 100 units at 4 p.m.

4/14/26. At 10:30 a.m. the blood calcium was 14.40 and the heart rate 50. Twelve cubic centimeters of magnesium sulphate were injected and the heart rate increased to 120. Blood calcium at 11:45 a.m. was 17.10. One hundred units of parathormone were injected at 11 a.m. and 100 units at 4 p.m.

4/15/26. At 9:45 a.m. the blood calcium was 24.38 and the heart rate 50. Nine cubic centimeters of the magnesium sulphate solution were injected and the heart rate immediately increased to 120. The blood calcium at 11 a.m. was 25.07.

4/16/26. At 9:25 a.m. the blood calcium was 20.14.

4/17/26. At 10:00 a.m. the blood calcium was 18.35. Ten cubic centimeters of magnesium sulphate was injected. The animal seemed less depressed after the injection.

4/18/26. The animal seemed stronger.

4/19/26. The animal was found dead in the cage at 9 a.m. The clotting blood from the thorax contained 17.75 mgm. of calcium per 100 cc.

Attention is called to the fact that the injection of the magnesium sulphate did not alter the calcium content of the plasma.

The above findings with regard to the heart and vessels are in accord with the observations of Zwaardemaker (1925), Steyus (1925) and Brull (1924–25). The two first-mentioned workers observed that the capability of the heart to stretch decreases as the calcium increases, while Brull showed that calcium solutions strengthened and slowed the isolated heart of the dog or rabbit, and caused a general vaso-constriction.

We have also observed this antagonism in the size of the veins. Under the influence of the hypercalcemia the vessels became so contracted as to make vene-puncture much more difficult. After the injection of the magnesium sulphate solution the vessels dilated and were more easily punctured.

Our observations confirm Collip (1926) in his record of the general depression, anorexia, vomiting, and final hemorrhagic infiltration of the gastric mucosa, with accompanying bloody exudation into the stomach, all induced by overdosage of the hormone.[1] After the animal begins to vomit bile and blood it does not recover, although it may die with the blood calcium returned nearly to normal. It has seemed to us that the first area of bloody exudation is the fundic mucosa, and we are engaged in determining the effect of moderate and severe hypercalcemia upon gastric secretion.

At the beginning of these experiments we hoped that medication by the magnesium sulphate injections would combat and overcome the toxic effects of the hormone. These expectations were not realized, probably because of the relatively transient effects of the magnesium sulphate as compared to the long duration of the hypercalcemia. We would emphasize the fact, however, that we have employed amounts of the hormone far beyond that required to alleviate the condition of tetany. We have never observed the toxic effects with a calcium level of 14 mgm. of calcium per 100 cc. of plasma. Therapeutic use of the hormone, then, does not, in our opinion, require an antidote. In cases of overdosage with the hormone we would not advise treatment with injection of magnesium sulphate solutions, because of the great toxicity of this salt.

[1] The pathological findings are being subjected to a detailed study by Dr. W. K. Hueper, of the Department of Pathology in this institution, and will be described in a separate report.

TABLE 3

Magnesium tolerance by animals with lowered blood calcium

DATE	ANIMAL NUMBER	WEIGHT	VOLUME OF 20 PER CENT $MgSO_4 7H_2O$ INJECTED	AMOUNT OF THE SALT PER KILO	Ca IN 100 CC. OF PLASMA	RESULTS OF THE INJECTION
		kgm.	*cc.*	*gram*	*mgm.*	
5- 1-26	M12	11.0	4.0	0.072	4.69	Relaxed and became comatose
5- 2-26	M12	11.0	5.0	0.081		Revived with injection of 5 cc. of calcium lactate
5- 8-26	M15	11.0	8.0	0.145	6.12	Asphyxial spasms. Revived with injection of 10 cc. of calcium lactate
5-17-26	M15	11.0	8.0	0.145		Asphyxial spasms. Recovery
4-27-26	M11	8.0	4.0	0.100	6.32	Relaxed. Recovery
5-28-26	M13	9.0	8.0	0.180	6.61	Died quickly
4-29-26	M12	11.0	4.0	0.081	6.71	Relaxed. Recovery
4-30-26	M12	11.0	5.0	0.091		Respiration failed. Revived with injection of calcium lactate
5-28-26	M14	9.0	8.0	0.180	7.80	Instant death
5-27-26	M14	9.0	4.0	0.091		Prostration. Recovery
5-17-26	M15	11.0	8.0	0.145		Asphyxial spasms. Recovery
8- 9-26	M30	15.4	15.0	0.196	6.85	Asphyxial spasms. Died in 3 minutes
8-11-26	M31	10.5	10.0	0.190	7.85	Instant death
8- 3-26	M29	11.0	15.0	0.273		Died after asphyxial spasm. (3rd injection)

The magnesium tolerance of hypocalcemic animals is shown in table 3. In order to render these animals hypocalcemic they were parathyroidectomized, with one exception, animal M31. This animal was injected, while the blood calcium was 12.52, with 20 cc. of 3 per cent sodium oxalate solution. Thirty minutes later, when the blood calcium was fallen to 7.85 and when marked muscular tremors were observed, the 10 cc. of magnesium sulphate solution were injected with the fatal results noted. Before some of the injections of magnesium sulphate solution into the parathyroidectomized animals the blood analyses were omitted because previous analyses had shown the animal was hypocalcemic. In one or two instances the calcium analyses were not made, but these animals were in active tetany. So in no instance of the table can there be any question but that the animal was hypocalcemic. In all other respects the bleeding and injection were made as already noted.

The results show definitely that the hypocalcemic animal is less tolerant to magnesium sulphate than the normal one. Our observations show that the fatal dose of the hydrated salt is 0.14 to 0.19 gram per kilo for the hypocalcemic dog, as compared with 0.23 to 0.28 gram per kilo for the animal with normal blood calcium.

We have also noted that the heart of the hypocalcemic animal is atonic and dilated. While no measurement of blood pressure were made, and none are available, it is our opinion that the blood pressure is elevated in moderate hypercalcemia and lowered in hypocalcemia.

The hypocalcemic animal responds to very small doses of magnesium sulphate. Small, non-fatal doses usually relax the animal from the condition of active tetany, and even render the animal comatose. Larger, non-fatal doses often result in respiratory failure and asphyxial convulsions. The respiratory center of the parathyroidectomized dog seems to be much more vulnerable to magnesium sulphate than that of the normal or hypercalcemic dog.

We wish to call attention to the fact that three of the animals appear in each of the three tables. These are M13, M14, M30. Each one of these was injected with the solution of magnesium sulphate while normal, hypercalcemic, and while hypocalcemic. Reference to the tables will reveal the fact that the hypercalcemic animal easily bore the injection which was nearly fatal when its blood calcium was normal, while the same animal did not tolerate and survive the same, or a reduced, amount while hypocalcemic. Animals M29 and M31 appear in tables 1 and 3 and experiments with them lead to similar conclusions. In this way we have made a series of five animals controls upon themselves, varying only the magnesium dosage and the blood calcium of one animal. We believe that we have thus eliminated the question of the individual resistance of the animal to magnesium sulphate injections. Any tolerance developed by

these animals, as suggested for the normal animals of table 1, would be exerted in a direction to oppose the results of these single control animals.

CONCLUSIONS

1. The fatal dose of rapidly injected 20 per cent solution of hydrated magnesium sulphate for the dog with normal blood calcium is 0.23 to 0.28 gram per kilo.

2. Dogs with hypercalcemia, induced by the parathyroid hormone, tolerate 0.30 to 0.32 gram of magnesium sulphate per kilo and are killed by about 0.35 gram per kilo.

3. The dog with hypocalcemia induced by parathyroidectomy or oxalate injection is killed by 0.14 to 0.19 gram of magnesium sulphate per kilo.

4. The toxicity of magnesium sulphate for the dog is closely proportional to the calcium content of the blood. Non-fatal injections of the solution of magnesium sulphate affect the hypercalcemic dog less than the normal one, and affect the hypocalcemic dog more than the normal one.

5. The parathyroid hormone exerts a marked effect on the heart and vascular system. This effect is antagonized by the injection of magnesium sulphate solution.

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THE HEAT PRODUCTION IN SMOOTH MUSCLE

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1. Early experiments. In the first communication on this subject (1914) I was unable to report a positive heat production accompanying contraction in terrapin's urinary bladder muscle. Soon thereafter Bernstein (1914) reported a rise of temperature accompanying contraction in a ring of frog's stomach muscle. But in some of the experiments of both these investigations the greatest source of error was the heat produced by the stimulating current itself. For as is well known this current in order to evoke contraction when applied directly to smooth muscle must be of much greater strength than is required for cross-striped muscle. For example, in my experiments the measurements showed that the stimulating current alone raised the temperature of the bladder muscle from a thousandth to a hundredth of a degree C. In cases of direct stimulation, therefore, if there is heat of muscular origin, even though it is as much as in the cross-striped muscles, it will be heavily masked by the heat of electrical origin. Obviously better results should be gotten by stimulating smooth muscle through the attached nerve. Although its isolation is much more difficult than the classical gastrocnemius-muscle-sciatic-nerve preparation, the cardia of the terrapin's stomach, isolated together with its branches of the left vagus nerve, has proved to be a smooth muscle-nerve preparation that could be used in these experiments. As an illustration of the performance of one of these surviving preparations a reproduction of an original drum record is submitted as figure 1. (See Addenda, 2.)

Notwithstanding the difficulties of obtaining ideal conditions for an experiment, my first results with this preparation demonstrated beyond a doubt that a rise of temperature accompanies the mechanical response to stimulation through the nerve. This result was briefly reported at the annual meeting of The American Physiological Society in December, 1919. The method used and some greater detail of the results of these earlier experiments may now be given.

To observe the temperature change a highly volt-sensitive d'Arsonval galvanometer was used in combination with a sixty-junction thermopile of the flat coil or insert type. The wires of the latter were 0.12 mm. in diameter and were wound on a flat plate of hard rubber. The thermopile

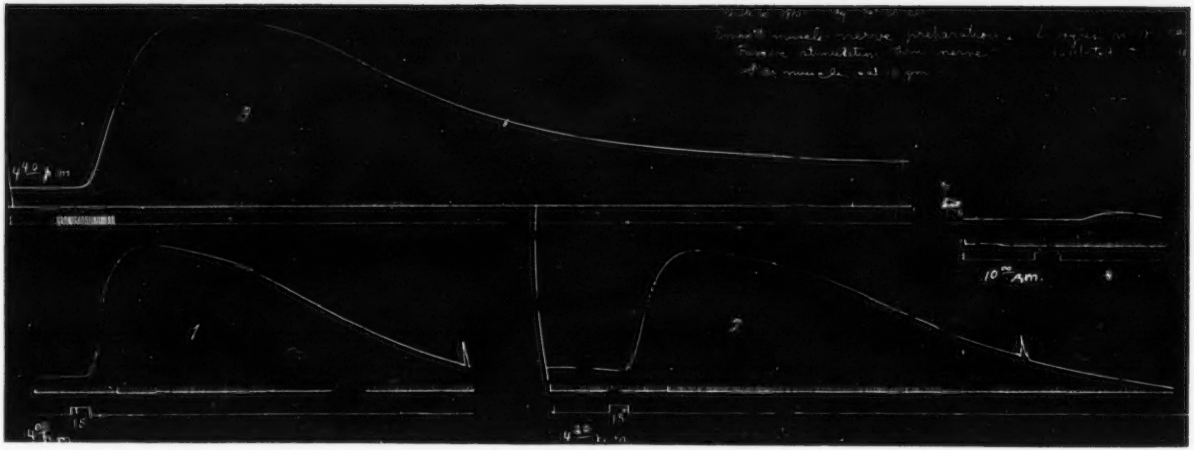


Fig. 1. Reduced ½ from the original. Myograms of a smooth-muscle-nerve preparation,—left vagus and gastric (cardia) sphincter. Faradic stimulation of nerve; time, in seconds; isotonic lever, weighted with *ca* 10 grams. The myograms, nos. 1, 2, 3 and 4, were taken at intervals as indicated in the record, at 4:00, 4:20 and 4:40 of the afternoon of iii/2/15, and again at 10:00 of the following morning.

was inserted into the suspended stomach ring, the tissues of which completely covered the warm junctions. But when the muscle contracted the "ring" took on a more circular shape and thus tended to pull itself away from the thermopile junctions. To avoid this, external supports had to be attached. If the frame of the thermopile had been given the shape of a well-filled wallet or a bolus of food (a flattened cylinder was later adopted) the junctions would have remained in better contact with the tissues in

TABLE 1

*Smooth muscle in the isotonic contraction**

NUMBER OF THERMO-MYOGRAM	STIMULATING CURRENT		THE MECHANICAL RESPONSE			GALVANO-METRIC DEFLECTION AT MAXIMUM	H/T*
	Secondary coil	Time of	Latency	Duration shortening phase	Duration of maintained tension†		
	cm.	*sec.*	*sec.*	*sec.*	*sec.*	*mm.*	*cal.* $\times 10^{5}$
1a	8	24	33	30	—	65	35.0
2a	8	21	48	24	60	60	32.3
2b	spontaneous contraction††		—	105	150	50	26.8
2c	4	36	54	24	90	50	26.8
2d‡	2	48	48	57	24	50	26.8
2e	0	36	0	21	>150	50	26.8

* *The experiment of xii/i/19.* The load constant at 20.7 grams; weight of muscle layers only 2.7 grams, of whole stomach ring, 5.35 grams. The galvanometer deflection of 1 mm. was equivalent to 2.5×10^{-5} deg. C. The ratio, H/T is the heat production per gram muscle per gram weight lifted, in calories found by the equation, $\frac{H}{T} = \frac{\text{galv. defl. in mm.} \times 2.5 \times 10^{-5} \times .83 \times 5.35}{20.7}$. See derivation of this, p. 735.

†† This tendency to go into spontaneous contractions makes it impossible to compare the mechanical response with any characteristic of the external stimulus applied; one cannot be sure in any case that there is not a summation of the intrinsic spontaneous stimulus and the external stimulus applied to the nerve trunk.

† The relaxation period was indeterminate; the muscle often contracted and could only be lengthened again by a stretching weight.

‡ The contraction was here greatest, the actual shortening of the flattened muscle ring being very nearly 1 mm., or 7 per cent of its length, the anacrotic limb of the curve indicates a sort of summation.

action without the external support. Another difficulty was the fact that the mucous coat of the preparation came into direct contact with the thermopile junctions if the surfaces of the stomach ring were kept in their normal positions. If the ring was turned inside out this brought the muscle coats in directer contact with the junctions, but in the process there was great danger of tearing some of the delicate strands of the nerve, and finally, of the pressure of the muscle against the thermopile frame acting as a block to the nervous impulses.

The determination of temperature rise was based upon the theoretical performance of thermopile, and the deflection of galvanometer to a known current, the sensitivity of the instruments being such that 1 mm. deflection of the galvanometer spot on scale was equivalent to 2.5×10^{-5} degrees C.

The contractions of the muscle were evoked by a tetanizing current (as shown in fig. 1) the strength being varied from just minimal to maximal, the duration from 16 to 28 seconds. In one of the experiments spontaneous contractions occasionally appeared; the heat production in these spontaneous contractions, it was of interest to observe, was in all respects quite like that observed when the contraction was evoked by tetanizing the vagus. Stimulation of the vagus thus, as one would expect, seemed only to arouse impulses in the final motor neurones of the

TABLE 2

*Smooth muscle in isometric contractions**

NUMBER OF MYOGRAM	STIMULATING CURRENT		OBSERVED TOTAL TENSION	THERMAL RESPONSE		
	Secondary coil	Tetanus		Deflection of galvanometer	Temperature rise in deg. C $\times 10^5$	H/T $\times 10^3$†
	cm.	*sec.*	*grams*	*mm.*		
I-2	8	24	37	310	776	64
II-3	6	28	51	330	825	49
III-1e	5	18	32.5	280	700	66
III-1f	6	21	32.5	270	674	63

* *From the experiment of xii/6/19.* The sphincter is everted and pulls against an isometric lever; temperature of muscle chamber 20°C.; weight of stomach ring 3.7 grams; weight of muscle 1.9 grams; 1 mm. deflection of the galvanometer equals 2.5×10^{-5} deg. C.

† The H/T values in table 2 again are gotten by using the general equation given on page 735.

preparation that were similar to those of spontaneous origin, the strength and duration of which showed little relation to the strength or duration of the tetanizing current itself. The results of the two experiments reported in the preliminary communication (1920) have now been reduced to an H/T ratio, according to the method described on page 735, and appear in tables 1 and 2. The load placed on the one muscle contracting isotonically was great enough to prevent undue shortening. In the muscle contracting isometrically the actual shortening was never more than 1 per cent of the length of the muscle ring. From the observations in tables 1 and 2 it would appear that the value of the ratio, H/T, for smooth muscle is many times that found for muscles of the cross-striped varieties in the single twitch. (For summaries of the latter data see my papers of 1922 and 1926, my paper with Gemmill, 1925, also table 6 of this article.)

What can be the cause or causes of this great divergence of results? To answer this question let us first examine the possible sources of error.

2. Sources of error. In the first place it must be pointed out that the time to maximum heat production, although it exceeds the time to maximum contraction as it does in other muscles, still in smooth muscle it is of a much greater absolute period, the period lasting from 24 seconds to more than a minute. Now this period is long enough to include most if not all of the heat of the recovery period in the cross-striped muscle and may thus account for some of this excess heat, but surely not for the whole of the temperature rise observed. There must be some other factor or factors involved.

Smooth muscle does not function well under rigidly isometric conditions; it becomes rapidly fatigued and soon refuses to respond at all. For this reason the muscles have been allowed to shorten a little in all the so-called isometric as well as in the isotonic contractions. This fact suggests errors of several kinds,—slipping of thermopile junctions over muscle surfaces; and, if the thermopile coil is moved in space, earth induction, and exposure of the thermopile to strata of surrounding medium of different temperature in cases where the medium is stationary. Furthermore there is the possibility, as already pointed out, that the glands of the mucous coats of the stomach preparation may also be stimulated to activity and thus give rise also to heat. Let us take up these items one by one.

a. Heat from glandular tissue. After a careful consideration of the possibility of heat arising from this source I have decided the amount thereof, if any at all, is negligible, and for the reasons 1, the glandular mass in the region of the cardiac sphincter of the stomach is small compared with the amounts of this tissue in other regions of the organ; 2, that the handling of the preparation and the constant contact irritation of the thermopile after mounting would soon result first in a constant, rather steady, output of heat and finally in complete fatigue of the glands from which no further stimulation could arouse them.

b. Slipping surfaces. The constant care that has been exercised during many years' experience to insure against slipping of thermopile junctions leads me to exclude this factor in my experiments as a possible source of error.

c. Earth induction. As to currents arising in the thermopile circuit it is obvious that the movement of the coil will be no faster than that of the contraction of the muscle. This in smooth muscle is so slow that current from induction will be exceedingly small. In his discussion of this subject Bürker (1905, p. 223) says that, so far as the movement is purely vertical and free from angular rotation currents of earth induction during the movement of the thermopile need not be taken into account.

d. A stationary medium in the muscle chamber. There is, however, another current that arises in the coil. I refer to the current that Bürker (1905, p. 224) believed to be the real source of the current that Blix regarded as currents of earth induction. If the thermopile coil is moved vertically in the mucle chamber and the medium filling the space of the chamber is stationary, then, the upper strata being probably warmer and the lower strata colder, the thermopile junctions will experience a change of temperature and a corresponding thermoelectric current will be set up. So long as the movement of the coil is slight and of relatively short duration this thermoelectric effect will be small, if the movement be large and the duration small, or the movement small and the duration long, then the thermoelectric effect will be considerable; if both the extent and the duration of the movement be large then the thermoelectric effect will be very large. When one begins to work with smooth muscle on a thermopile coil in a stationary medium of the muscle chamber one has just the conditions that give rise to thermoelectric currents of this sort that are of disturbing magnitude. Smooth muscle must be allowed to shorten more than other kinds of muscle when stimulated to contraction and when it finally goes into contraction it produces a movement that covers a longer period of time.

e. Experiments on moving a thermopile coil in a stationary medium. To put this matter to a test as to what the negligible limits may be, I set up experiments using the same instrumentarium that I use for smooth muscle experiments. With no muscle on the coil but a slender silk thread attached to it the coil was shut up in the muscle chamber and, by means of the silk thread, attached externally to a muscle lever. The coil hung in the chamber in the same position as it does in a muscle experiment. In one series of observations the coil and chamber were free from moisture, in another wet cotton-wool pads, of the same shape and mass as the tissues in a muscle experiment, were attached around the thermopile junctions. The coil was then closed up in the chamber and one waited for a temperature equilibrium to establish itself. When this took place it was noted at once that movement of the muscle lever, which now moved the coil vertically in the muscle chamber, was accompanied or rather followed, after a marked latent period, with marked deflection of the galvanometer, which increased with increase of extent and duration of the movement of the thermopile coil.

When the coil was lowered (and this is the direction of coil movement in a muscle experiment) the galvanometer deflection was always in the direction produced by a warming of the warm junctions or a cooling of the cold junctions. The wet pads over the warm junctions were made thicker than those over the cold junctions in order to have the conditions conform with the muscle experiment. This enabled heat to be with-

drawn from the cold junctions more rapidly than from the warm junctions, as the coil was lowered into the colder air, and since the cooling of the cold junctions has the same effect as the warming of the warm junctions the deflection of the galvanometer in the direction caused by a "positive heat production" was inevitable. The quantitative results of this experiment appear in table 3, the galvanometer deflections being put in terms of equivalent temperature changes. It should be added that the coil displacement was effected in this experiment by hanging weights of different dimensions on the muscle lever. This produced the desired movement of the coil suddenly, and the displacement so effected was maintained until the galvanometer deflection had reached its maximum.

Under D in the table is set down the actual maximum vertical movement of the coil in terms of millimeters, under Θ is placed the corresponding maximum change of temperature as recorded by the galvanometer. Under the heading Θ/D is put the temperature change per millimeter of coil displacement. Under t' I have added the times required for the galvanometer to reach its maximum deflection and under Θ/Dt' the temperature changes per millimeter coil displacement and per second of the duration of the galvanometer response. The latency of the galvanometer (L) response appears in a separate column.

It is of interest to note that the latency of the galvanometer response following coil displacement is relatively a long period, ranging from 25 to 65 seconds. This indicates that the coil displacement in a muscle experiment must last for periods approximating these periods, or for longer periods, in order that the colder air of the chamber may have its full effect on the thermopile. The duration of contraction of smooth muscle here reported covered periods from 60 to 172 seconds; the time to maximum galvanometer response lasted from 24 to 57 seconds, the whole of the response covered from 78 to 259 seconds. The actual shortening of the muscle ring was from 0.5 to 2.5 mm. It is clear therefore that if heat production of muscle is observed under these conditions then an error of large magnitude enters into the observation due to the vertical displacement of the thermopile coil in the stationary medium of the muscle chamber.

f. A correction factor for coil displacement. The importance of a correction factor for vertical coil displacement, where the medium filling the muscle chamber remains stationary, thus becomes obvious. In searching for such a factor I have considered both the ratios, Θ/D and Θ/Dt', as exhibited in table 3, in which D is the actual maximum shortening of the muscle preparation in millimeters and t' is the duration in seconds from the beginning to maximum of the galvanometer deflection. That time as well as distance is important appears when one compares these two ratios. Those for Θ/D in their extremes vary as much as +67

per cent and −46 per cent, those for Θ/Dt' vary roughly ±20 per cent, from their respective averages. The ratio Θ/Dt' thus turns out to be the better constant; its average value is 0.64×10^{-5} degrees C. Is it permissible to apply this figure as a correction factor to the observations of temperature changes occurring during contraction of smooth muscle preparations set up and operating with the same apparatus and the same conditions?

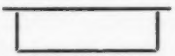
Without entering into a discussion of the matter let it suffice to say that I have done so, and in the following manner. In the first place in the muscle experiments the average amount of coil displacement per contraction has to be determined. In my tests above on the coil without the muscle, the displacement desired in each case was sudden and complete;

TABLE 3

On the effect of thermopile coil displacement, vertically in the muscle chamber

NUMBER OF TRIAL	ACTUAL VERTICAL DISPLACEMENT OF COIL* (D)	LATENCY OF GALVANOMETRIC RESPONSE (L)	MAXIMUM GALVANOMETRIC DEFLECTION	MAXIMUM GALVANOMETRIC DEFLECTION IN DEG. C × 10^5 (θ)	DURATION OF GALVANOMETRIC RESPONSE TO MAXIMUM (t')	$\frac{\theta}{D}$ DEG. C × 10^5	$\frac{\theta}{Dt'}$ DEG. C × 10^5
	mm.	*sec.*	*mm.*		*sec.*		
a	0.454	65	8.2	21.1	62	46.4	0.75
b	0.756	25	28.0	72.3	140	95.6	0.67
c	0.278	67	15.5	40.0	198	145.0	0.73
d	0.480	63	14.6	37.7	138	78.6	0.57
e	0.681	27	18.1	46.7	147	68.7	0.47
Average.....						86.86	0.64

* The muscle-lever used here is a calibrated "isometric lever;" from the displacement of the writing tip on the record one calculates (in the usual way) the actual displacement at the coil from its known point of attachment to the lever.

the time-distance or area of this displacement therefore may be represented by a two dimensional rectangle thus,  , whereas the time-distance or area of the displacement produced by the contracting muscle is represented by a triangle thus,  . The upper horizontal lines of these diagrams represent the base line in each case, the triangle and the rectangle depending from the base line in each case represent the areas of displacements. The area of the triangular figure being half that of the rectangular figure, its average amount of displacement is therefore one half of the maximum vertical movement. But since the contraction curve of the muscle is somewhat concave to the base line, I have arbitrarily taken $\frac{7}{12}$ of the actual shortening as the

average displacement during the whole of the contraction period. The correction factor then becomes $\frac{5}{12} \times 0.64^{-5}$ degrees C. per second of the duration of movement and per mm. of displacement.

g. Drifting level of resting heat production. When so-called temperature equilibrium has been reached in the muscle chamber there often

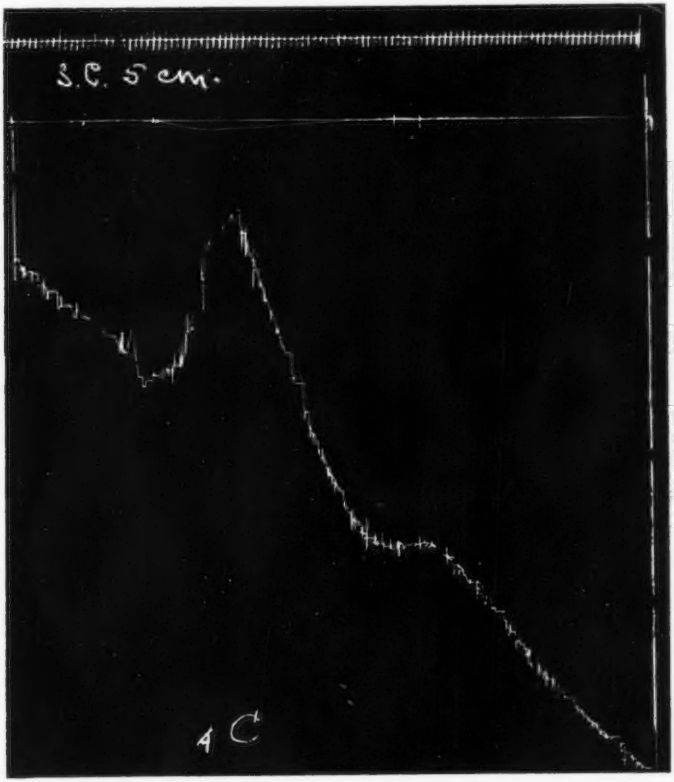


Fig. 2. Reduced $\frac{4}{5}$ from the original. From the experiment of v1/24/26. A thermomyogram of a surviving smooth-muscle-nerve preparation (the vago-cardia sphincter of the terrapin's stomach) in response to faradization of the nerve. The upper trace-line is the time trace in 3-second intervals and serves also to signal stimulation. The middle trace-line shows the mechanical response (isometric lever), the lower trace-line the thermal response, or thermomyogram, of the muscle. Upward movement of thermomyogram indicates a rise of temperature of the muscle (warming of "warm" junctions). The down-stroke of the mechanical trace-line indicates rising tension; up-stroke, relaxation of muscle. The thermomyogram is superimposed upon a falling shift, or a "decreasing resting heat production."

Fig. 3. Reduced $\frac{2}{3}$ from the original. Same as figure 2. Trace-lines explained in legend to figure 2. Thermomyograms superimposed upon a rising shift or "increasing resting heat production." (See text, page 727.)

sets in during an experiment a troublesome shift of the galvanometer spot while the muscle is at rest. This indicates a rising or a falling level of "resting heat production" in the muscle. The change of resting heat level is only apparent however. The real cause of the shift is due to slight slow changes of the temperature of the medium filling the muscle chamber and to unequal rate of heat conduction (unequal heat insulation) of the two sets of thermopile junctions. If this shift in the resting heat level is at a steady and not too fast a rate it does not vitiate the thermomyogram. For if both the angle of shift and its rate are recorded, both before and after the muscle is made to contract, then the intervening thermomyogram becomes simply superimposed upon a continuous linear shift (see thermomyograms in figs. 2 and 3). The base line of the thermomyogram is the straight line connecting the resting heat levels before and after the thermal response to stimulation. Comparison of records when the resting heat level is at zero degrees angle and when it is, say at 20 degrees angle, shows no other appreciable differences.

3. THE METHOD AND PROCEDURE OF RECENT EXPERIMENTS. In renewing the experiments with the terrapin's gastric sphincter one of the first things to do was to construct a thermopile frame to conform with the shape of the hollow space that was to receive it. The flat coil thermopile can be inserted into a stretched and flattened stomach ring, but when the ring contracts it tends to become more circular. If the stretching tension on the muscle is slight then the tissue tends to pull away from the thermopile junctions; if the tension is great enough to prevent this then the muscle becomes quickly fatigued and refuses soon to respond at all. To meet this situation I wrapped a chain of thermopile wires on a block of cork that was fashioned into the form of a somewhat flattened cylinder. This block in cross-section is nearly an ellipse, with the short axis 6 mm., the long axis 22 mm., in length. By the wrapping of the wires the warm junctions fall along the points where the short axis bisect the periphery and the cold junctions fall along the points where the long axis bisect the periphery of the successive (imaginary) elliptical cross-sections of the block. When now the stomach ring is slipped over this wallet-shaped thermopile it covers the warm junctions completely and upon contracting hugs them more tightly rather than less, much as it would a bolus of food.

A highly volt-sensitive d'Arsonval galvanometer again was used the half period of which when critically damped is about 12 seconds. It will be noted in table 1 that the duration of time required to maximum temperature rise in no case was less than 24 seconds; the period of the galvanometer therefore was sufficiently short. The galvanometer deflections again were recorded by the method I described in 1922. By this method the mechanical responses together with time and stimulating

signals are simultaneously recorded with the thermal responses upon a single smoked drum. The muscle lever trace-line on the drum record shows the relative positions of the muscle lever successively at zero and initial tensions as well as the changing tensions during the muscle contraction; the galvanometer trace-line shows the deflection of the galvanometer to the calibrating current, and sometimes its movements as the shift is made from galvanometer null-point to resting heat production as registered by the thermopile, that is, the whole of the galvanometer record upon switching from calibrating to thermopile circuit. Examples of records of this kind will be found in figures of the paper of 1922.

The isolation of the cardia sphincter with the nerve intact is a tedious procedure. I have used terrapin having body length from 20 to 25 cm. After destroying the brain (crushing the head with a single blow with a blunt instrument is a quick and satisfactory way) one fastens the body on its back in a holder and removes the plastron. To expose the nerve and the cardia one may begin either with the cephalad end of the vagus or with the stomach end of the preparation. Freeing the vagus down to the point where the recurrent laryngeal leaves it is quickly done but from here on to the region where the branches pass out to the visceral organs great care must be exercised. If the lungs of the animal are not already inflated this should be done and the bronchi tied off; this keeps the field of operation well up out of the extreme depths of the body cavity. The usual procedures to maintain a bloodless field must be rigidly followed. If these precautions are taken the fine branches of the vagi supplying the cardia of the stomach are soon found sharply outlined in the clear fluids of the cavity. The branches of the left vagus are traced back to the larger strands that give rise to them where it is found they twist around the pulmonary blood vessels and bronchus of the left side. This is the most difficult part of the dissection. The nerves must be carefully freed from connective tissue that holds them to the walls of the blood vessels and bronchus; double ligatures are then laid around the latter structures, whereupon they are severed and their cut ends gently lifted out of the encircling nerve branches. Finally the nerve filaments to the stomach that lie along the base of the esophageal wall are freed. Tetanization now of the left vagus trunk will test the functional integrity of the fibers supplying the cardia sphincter; this organ if responding to the nerve stimulus will be seen as an annular elevation separating gullet from stomach and may be felt by the fingers as a hardened annular mass. The outlines of the sphincter now having been made out, one proceeds at once to remove it by sectioning with strong, sharp scissors a few millimeters below and a few millimeters above, keeping an eye on the whereabouts of the precious nerve and its attachments. The stomach ring after isolation, on account of the contraction of its few longitudinal fibers, will have

a lesser width, measuring about 8 to 10 mms. The whole time of the operation including mounting the preparation in the muscle chamber lasts from one and one-half to two hours. But if one has the patience one may become skillful enough to preserve the nerves uninjured and finally to have a surviving smooth muscle-nerve preparation that, I believe, will be found useful for many studies in pharmacology as well as in physiology.

As to the muscle chamber itself and the method of maintaining a constant temperature this has been described and shown in diagrams by Gemmill (1926) to which the reader is referred. Here it should be emphasized that double dewar "food-jars" are used. For temperatures to be kept below room temperatures the jars are chilled with ice while the preparation is being dissected out. When the muscle is ready to be put in the chamber, the ice is entirely removed from the inner (muscle) chamber and the space between the inner and outer chamber walls is packed entirely with cracked ice, if a temperature of 0 to 3°C. is wanted; but if higher temperatures are wanted then this space is packed below, and in the lower reaches, with loose dry cotton wool and the ice is put only above. The relative proportion of the space packed with wool to space packed with ice is varied according to the temperature wanted. The idea in putting ice only in the upper portion of the space between the two chamber walls and loose wool pads in the lower, is to insure an equalization of temperature of the whole space by the convection air-currents that arise by this arrangement. After the muscle is mounted properly in the inner chamber the whole is covered over with generous layers of cotton wool.

While this method does not insure an ideal equilibrium of temperature in the inner chamber suitable for the thermopile, it nevertheless does insure a desirable temperature *level* for nerve-muscle preparations. For example, in the experiment about to be reported the temperature of the muscle-chamber at 1:45 p.m. was 14.10°C. and at 5:10 p.m. it was still 14.05°C. By 5:45 p.m., the ice was pretty well used up and the temperature began to rise, being then 14.80°C. But the packing, being in the outer chamber space, can be renewed easily without serious delay of the experiment.

The preparation having been mounted in the chamber and the outer packing completed, the muscle is connected to the muscle lever, and through the proper leads to the necessary electric circuits. While waiting for temperature equilibrium to be established within the muscle-chamber, as Bürker (1908) advises, one assembles the materials and apparatus still needed for recording observations, inspects, tests, adjusts, and calibrates the performance of every unit in the instrumentarium, electrical keys, circuits, shunts, resistances, galvanometer, calibrating potential, stimulating current, etc., etc., preparatory to the final taking of records.

In what follows I shall report the procedure and results of a recent experiment with the cardia-vagus preparation, that of June 24, 1926.

4. A CRUCIAL EXPERIMENT. The vago-cardia sphincter preparation was isolated, mounted in position on the thermopile, and the muscle chamber packed and closed by noon. By 1:45 p.m. records were already taken, and from then on, from time to time, covering a period of nearly nine hours. Smooth muscle at 14°C. acts slowly, but during this period fourteen myograms with their accompanying thermograms had been recorded. One of these being incomplete was discarded and the remaining 13 records were analyzed; their results are gathered up in tables 4 and 5.

In table 4 most of the durations are exhibited, but also the stimulating current which is given in terms of coil distance in centimeters and dura-

TABLE 4

Experiment of vi/24/26.—Heat production in smooth muscle

HOUR AND NUMBER OF MYOGRAM	STIMULATING CURRENT		LATENCIES		DURATIONS	
	Distance secondary coil	Time of stimulation	(a) of mechanical response	(b) of thermal response	(a) of tension rise to maximum	(b) of whole thermal response
	cm.	*sec.*	*sec.*	*sec.*	*sec.*	*sec.*
1:45-a	7.3	14.0	15.0	18.0	24	78
2:00-b	7.3	18.0	22.0	24.0	27	149
3:20-c	7.0	21.0	18.0	20.0	29	112
3:48-d	6.0	28.0	22.0	24.0	26	101
4:50-e	5.0	19.0	15.0	27.0	30	124
5:10-f	4.0	1.0	18.0	18.0	29	121
5:30-g	4.0	1.5	19.0	19.0	22	157
5:48-h	4.0	1.0	16.0	22.0	30	
6:43-i	4.0	0.5	10.5	10.5	18	250
6:58-j	4.0	0.5	7.5	9.0	19	168
7:12-k	3.0	0.3	12.0	12.0	17	159
7:33-l	3.0	0.6	6.0	8.5	18	183
9:18-m	3.0	2.0	5.5		23	

tion of stimulus in seconds as applied to the nerve. The absolute strength of the current here is of no importance. But the strength of current (the source being a Porter inductorium) was varied from minimal to maximal in the usual physiological sense of these terms. By latent period here is meant the times from moment of onset of faradizing the nerve to the moment of beginning mechanical response (movement of isometric lever) in the one case, and to the moment of beginning of galvanometer response in the other. The times of mechanical and thermal responses themselves do not include the latent periods. From these it will be seen first that the duration of the mechanical responses were not correspondingly prolonged for the longer, or shortened for the shorter, periods of tetanization. A period so brief as 0.5 second of stimulation of the stronger currents evoked

a response two to three times the period of response evoked by the weakest current when applied for 14 seconds. All this agrees with observations that have often been made on such smooth-muscle organs *in situ*. Here again it is obvious that the impulses set up in the vagal fibers stimulate the ganglia in the stomach nerve-plexuses and these in turn create impulses

TABLE 5

The experiment of vi/24/26.—Heat production in smooth muscle

NUMBER OF THERMOTOGRAM	WHOLE TIME OF MECHANICAL RESPONSE (M)	DURATION FROM MAXIMUM MECHANICAL TO BASE LINE (R)	MAXIMUM TENSION OBSERVED (T)	ACTUAL MUSCLE SHORTENING (D)	TIME TO MAXIMUM GALVANOMETRIC DEFLECTION t'	OBSERVED MAXIMUM GALVANOMETRIC DEFLECTION IN DEG. C × 10^3	CORRECTION FACTOR TO MAXIMUM TEMPERATURE RISE IN DEG. C × 10^3	OBSERVED MAXIMUM TEMPERATURE RISE CORRECTED DEG. C × 10^3 (H)	$\frac{H}{T}$ CAL. × 10^3†	$\frac{H}{TR}$ CAL. × 10^4	$\frac{H}{TM}$ CAL. × 10^4
	sec.	*sec.*	*grams*	*mm.*	*sec.*						
a	60	36	6.11	0.5	29	25	5	20	3.3	0.91	0.54
b	81	54	7.04	0.7	34	48	8	40	5.7	1.05	0.70
c	81	52	8.41	1.2	30	51	13	38	4.5	0.87	0.56
d	71	45	8.66	0.8	42	44	12	32	3.7	0.81	0.51
e	120	90	8.04	2.0	24	89	18	71	8.8	0.97	0.73
f	134	105	8.26	1.8	40	105	27	78	9.5	0.90	0.70
g	130	108	8.41	1.5	29	107	16	91	10.8	1.00	0.83
h	126	96	8.81	1.5	47	88	26	62	7.1	0.73	0.56
i	172	154	9.56	2.5	48	160	44	116	12.1	0.78	0.70
j	148	127	9.72	2.2	57	138	47	91	9.5	0.74	0.63
k	137	120	9.19	2.5	44	115	41	74	8.0	0.67	0.57
l	147	129	9.20	2.5	41	134	38	96	10.4	0.80	0.70
m	106	83	7.04	1.7	35	70	23	47	6.7	0.81	0.63
Average.....			8.36						7.7	0.85	0.64

† Substituting the numerical values for this experiment,

$$\frac{H}{T} = \frac{\text{obs. temp.} \times 1.215 \times 0.825 \times 0.63}{0.63 \times \text{obs. tension}}$$

in which the figures 1.215, 0.63 and 0.825 represent respectively the weight of the stomach ring, the weight of the muscle layers only of the ring, and the specific heat of all the tissue. The number for the specific heat as determined for muscle has been assumed to be the same for the other tissues of the stomach ring. The tension was recorded by a calibrated isometric lever.

in post-ganglionic fibers the strength, frequency and duration of which we do not know. The latencies exhibited in the tables obviously include in each figure a number of delays, preganglionic, synaptic, post-ganglionic, myoneural and muscular!

In table 5 the tension and thermal responses are chiefly considered,

first by themselves then in their relation to each other and finally in their relation to the durations of the muscle's activities. It will be noted that I have subtracted the probably equivalent thermal effect on the galvanometer deflection produced by the thermopile coil being lowered vertically when the muscle contracted. The amount of this correction appears in each case under the heading "Correction factor. . . ." in the seventh column; this is subtracted then from the apparent temperature rise of the muscle as recorded in the sixth column. The actual maximum temperature rise of the muscle finally appears in the eighth column under H. This maximum temperature rise of the preparation is taken as a measure of the total heat production of the muscle including recovery heat; it was also the measure I used in determining total heat for Limulus heart (1926). This method cannot be used for muscles producing relatively high initial heats, or whose recovery heat is completed only after a period of six minutes and longer after the stimulus has ended, as is the case with frog's sartorius and gastrocnemius muscles. But with tissues where the amount of heat production is small compared to the mass of tissue, and it develops at a more regular rate, the loss of heat, before its full effect is registered as temperature rise, is negligible.

These conditions are met with when one uses thermopiles such as I have used, and muscles such as those from hearts of the cold-blooded animals and smooth muscle organs. In these cases the maximum deflection of the galvanometer remains small, and the whole duration of the deflecting (including return to zero) rarely exceeds 3 minutes. As is seen in table 4 the duration of the whole thermal response in one case only exceeded 6 minutes and that only by 10 seconds, whereas the durations for all the other cases varied between 78 and 183 seconds. The maximum extent of galvanometer deflection likewise was small, registering a temperature rise of 0.00115°C. for the maximum case, and between 0.0002°C. and 0.00096°C. for the other cases.

The relation of heat production to tension set up by the smooth muscle appears in the column headed H/T in table 5. It varies greatly but its average value is 7.7×10^{-5} calorie. This is much smaller than the value of H/T gotten from the first experiments (tables 1 and 2) and the reason is clear. In the first place no correction for the vertical displacement of the thermopile coil in a stationary medium of the muscle chamber was made. This correction, as may be seen by comparing the figures in table 5, may be more than 35 per cent. For results given in table 2 the required correction no doubt will turn out to be much more, perhaps 50 per cent, in which case the observed rise of temperature in the four contractions would be halved, and the H/T ratios $\times 10^5$ for the muscle would be 10.5, 8.1, 10.8 and 10.0 respectively. This brings them within the range of the values for total heat of the muscles shown in table 6.

The method adopted for the calculation H/T for preparations such as are used in this experiment is as follows. In cases where the preparation consists partly of passive tissue both the whole weight of the preparation and the weight of the active muscle alone must be taken into account. To find then the heat production per gram muscle per gram tension exerted (H/T), since it is only the active muscle portion that produces both, the equation, $\frac{H}{T} = \frac{tsw_p}{\tau}$, should be used. In this equation H is the heat production and T the tension developed per gram active muscle; s is the specific heat of all the living tissues (0.83 may be taken); w_p is the weight of them, excepting the nerve trunk; t is the observed rise of temperature of the preparation and τ is the maximum (total) tension developed by the whole mass of active muscle. The weight of this muscle mass is represented below by w_m.

The derivation of the equation becomes clear when we consider first how we determine H, the amount of heat in calories produced by one gram of active muscle during the act of contraction. The maximum rise of temperature having been observed we may assume that the whole of the stomach ring has experienced this rise of temperature. The calories of heat per gram weight of the preparation necessary to effect the observed rise of temperature must be that rise times the specific heat of the tissues, or ts; and the total heat, this number times the weight of the tissues, or tsw_p. But we assume that only a part of the tissues, the active muscle fibers, has produced all the heat; so that one gram of the muscle must have produced tsw_p/w_m part of the whole; then, $H = \frac{tw_p s}{w_m}$. But the total tension also having been developed by the muscle only, the tension exerted by one gram of muscle is $T = \frac{\tau}{w_m}$. Then $\frac{H}{T} = \frac{tsw_p}{w_m} \cdot \frac{w_m}{\tau}$, or $\frac{H}{T} = \frac{tsw_p}{\tau}$, as given above. The values of these quantities for each case will be found in the tables excepting the constant ones, the weight of the preparation, which was 1.215 grams and that of the active muscle which was 0.63 gram, and the specific heat which was taken as 0.825.

It will be noted that the value of the ratio, H/T, for the terrapin's cardiac sphincter varies roughly from 3×10^{-5} to 12×10^{-5} calorie. This means that the heat produced by this smooth muscle of the stomach[1] upon being faradized through its attached nerve produces from 30 to 120 microcalories (millionths of a small calorie)[2] of heat per one gram weight of tension per gram weight of muscle.

[1] I use this repetition advisedly. One reviewer of my first communication (1920) thought it was a preparation of the heart!

[2] See footnote on page 739 for this term.

5. TOTAL HEAT AND INITIAL HEAT. It may be recalled now that this ratio of total heat to tension for spontaneously beating terrapin ventricle was found to vary between 24 and 174, or to have an average of about 105, microcalories[2] (1922); for a spontaneously beating Limulus heart the ratio averaged 115 in one case and in another 71 microcalories (1926). But for the skeletal variety of cross-striped muscle, when contracting in single twitches, this H/T ratio must be about 61 microcalories (frog's sartorius, (Hartree and Hill); and, if the latter's ratio of initial to total heat, 1:2.5, is correct, then the average value of H/T for the frog's gastrocnemius is probably about 70 microcalories. This last figure is based

TABLE 6

Comparing H/T ratios from various muscles

KIND OF MUSCLE	H_t/T CAL. $\times 10^5$	H_i/T CAL. $\times 10^5$
Smooth muscle, terrapin's gastric sphincter; H_i/H_t = 1/4.33	7.7	[1.8]
when H_i/H_t = 1/2.5,		[3.1]
Limulus heart (cross-striped tissue) °1	7.1	[1.6]
H_i/H_t = 1/4.33, °2	11.5	[2.8]
Terrapin auricles (mostly smooth muscle fibers)* H_i/H_t = 1/4.33	9.3	[2.1]
Terrapin's ventricle (1922)	10.5	2.7
Frog's gastrocnemius muscle, average of 5 experiments;		
°1, H_i/H_t = 1/2.5	[7.0]	2.8
°2, H_i/H_t = 1/4.3	[12.1]	
Frog's sartorius, mean of extreme observations (Hartree and Hill, 1924);		
°1, H_i/H_t = 1/2.5	6.1	2.5
°2, H_i/H_t = 1/4.3	[10.8]	

* This observation is taken, by kind permission, from a research from my laboratory by Dr. C. L. Gemmill, 1926, now in press.

on a calculation of the initial heat as observed by Snyder and Gemmill (1925). From table 5, however, we see that the average value of H/T for our smooth muscle is about 77 microcalories.

In table 6 I have summarized these average values for the various muscles, the total heat appearing under the ratio H_t/T and the initial heat under the ratio, H_i/T. The figures appearing in brackets have been calculated merely from their fellows that are not in brackets and that are the ratios of averaged observed heats (either initial or total) and their corresponding tensions. The ratio of initial to total heat for terrapin's ventricle as I pointed out (1923) must be about 1:4.33. In table 6 the values of the H/T ratios are also calculated on this basis as well as the 1:2.5 basis for the sake of comparison.

In view of the comparative slowness of the processes of smooth muscle,

both mechanical as well as thermal (see figures under columns M and R, table 5) I am convinced, as already stated, that we are dealing here with the total heat production rather than any small moiety thereof. The fact that smooth muscle organs do not act with all their fibers pulling together at once makes it unlikely that any so-called initial heat will be demonstrated in the single contraction for this tissue. Studies in the chemistry of smooth muscle indeed will have to be made; and then, if we once know the chemistry of the recovery processes, we may get at the chemistry and finally the heat changes during the period of rising tension. If we suppose for the moment that there is an initial heat liberated of the proportion to the total heat such as has been found for other muscle, then it is obvious that the value of H_i/T for smooth muscle probably will not be less than 1.8×10^{-5} calorie nor more than 3×10^{-5} calorie, as is provisionally indicated in table 6.

Returning now again to the observed figures on total heat of the various muscles in this table, one notes with no little surprise that the total heat of the smooth muscle preparation when reduced to a common standard of measure turns out to be as large as it is for skeletal muscle.

The ratios for the preparations of the terrapin's heart are distinctly higher, lying between 10.5×10^{-5} and 11.5×10^{-5} calorie for the single spontaneous beat. This will be dealt with later. The value for H/T, however, for the frog's sartorius muscle is 6.1×10^{-5} calorie and probably that for frog's gastrocnemius muscle is 7.0×10^{-5} calorie (both muscles in the single twitch). These are practically the same as the average ratio of H/T for the smooth muscle, as shown in table 5, namely, 7.7×10^{-5} calorie. This result does not appear to be in keeping with our present conceptions of the energy exchanges in active smooth muscle.[3] If, however, one compares the kind of contraction the stomach ring enters into when stimulated through its vagus nerve, that the response is more in the nature of a tetanus, a response to a cataract of self imposed impulses, if you will, rather than to a single impulse, as is undoubtedly the case in spontaneously beating heart muscles and as must be the case by the condition of the experiment with the skeletal muscles in single twitches, then one realizes that in table 6 we are comparing two different things,—the smooth muscle when thrown into prolonged contractions, and the cross-striped muscles when thrown into single twitches.

6. THE HEAT PRODUCTION PER SECOND OF THE WHOLE TIME DURATION OF THE CONTRACTION OF SMOOTH MUSCLE. The reader doubtless has been struck by the great divergence among the various individual values of H/T, for the smooth muscle as they appear in table 5. The extreme deviations are $\pm$ 57 per cent from the average of the 13 observations. The tensions

[3] For a brief review of the literature and discussion of the subject of energy exchange in smooth muscle see my first communication on the subject (1914).

do not vary quite this much, their extremes being ±47 per cent from the average. H/T, therefore, cannot be regarded in any sense as a constant proportion between H and T. Some factor other than tension must be involved. It has been known for a long time that a tetanized skeletal muscle experiences a greater temperature rise than does one stimulated with a single shock, and Hartree and Hill (1921) have shown a definite relation between the amount of heat production and the duration of the stimulus, between certain limits. If any corresponding relation holds also for smooth muscle then the duration of the mechanical response must be included in our considerations. But how can we utilize this factor in the present experiments? If Hartree and Hill's findings are correct then also the converse of their statement must be true for frog's sartorius muscle. If the amount of heat production is in part a function of the duration of the tetanus then the duration of the tetanus also in part must be a measure of the heat production.

Does smooth muscle behave like skeletal in this respect? If smooth muscle continues to produce heat *pari passu* with the duration of the mechanical response then the H/T ratio, other things being equal, would of course be large, and to reduce it to simpler terms one must be able to introduce a duration factor. Out of curiosity as to this matter I divided the H/T values (table 5) by the durations of the corresponding mechanical responses in each case, trying first the durations of relaxations only (R), and then the duration of the whole contraction period (period of rising tension plus relaxation, M). The results appear in table 5 under the rubric H/TR and H/TM. The values for both these ratios vary less among themselves than those for H/T, the extreme H/TR ratios showing a deviation of ±22 per cent from their average value, the extreme H/TM ratios varying ±25 per cent from their average value. This is a great improvement over the H/T ratios and over the percentage deviation of the tensions taken alone. Indeed both H/TR and H/TM approach as nearly a constant as has been obtained in work on heat production.

Which of these two ratios represents the more useful constant can only be determined by further investigation. The period of rising tension (C, table 4) represents a smaller fraction of time and doubtless also a less variable output of heat; R, the period of relaxation and recovery represents a greater fraction of the whole time and probably a greater variable heat output and ought, therefore, give a better constant. However, in what follows I shall direct attention only to the ratio containing the M factor, the whole of the time of the mechanical response.

It then appears from the ratio, H/TM, that when faradized through the preganglionic fibers of its motor nerve into (nearly) isometric contractions the cardiac sphincter of the terrapin's stomach produces an average

of about 0.64 microcalorie[4] of heat per gram weight of tension exerted per gram weight of muscle and per second of the whole duration of the mechanical response.

There is nothing in the literature on heat production of muscle that is strictly comparable to this result, arrived at in this way, not only because of the peculiar structure of smooth muscle but also because of the very indirect stimulation effected through the preganglionic fibers of the muscle. The total heat as well as the total time of the muscle response are taken as bases of computation. If one wishes to compare the smooth muscle result with skeletal muscle findings then one must have results based upon total heat and total time of the muscle action, including the recovery time. Hartree and Hill (1921) do not treat their data in this way. But if one does so treat their data it would appear that sartorius muscle at 14 degrees C. tetanized for two seconds produces about 52 microcalories heat when expressed as H/Tl, *l* giving the heat per unit fiber length instead of the unit weight of the muscle mass. Now the time for the recovery heat to be developed when skeletal muscle contracts with a single twitch is known to require a very few minutes, but still something more than one minute. If the average time of the tetanized sartorius is the same as the duration of the whole smooth muscle contraction, 116 seconds, then the value of H/TlM for sartorius would be only 0.45 microcalorie. From other studies of the same authors on total heat of sartorius, I have estimated from their H/Tl ratio that in the single twitch H/T has a value of about 61 microcalories per gram weight muscle per gram weight tension exerted (see my paper, 1926, and table 6 of this paper). If only 60 seconds are required for recovery in the single twitch, then one microcalorie of heat is produced per second; if 180 seconds are required then 0.33 microcalorie heat is produced on the average per second of the whole muscle cycle; that is, when M equals 60 or 180 then H/TM = 1.0 or 0.33 microcalorie.

It thus appears when the observations are all reduced to terms of a common standard of measure that the heat production of smooth muscle is not less than it is for skeletal muscle.

7. THE DATA ON CROSS-STRIPED MUSCLE REËXAMINED. Before going farther with this comparison of the different kinds of muscles and above

[4] The term "microcalorie" is not used here or in my previous papers as A. Fick (1882) used it and who meant thereby only 0.001 small calorie (1 millionth of a large calorie). We have already the specific designations Cal. and cal. for the large and small calorie. It seems to me, therefore, that since "cal." already designates 0.001 Cal., let millical. = 0.001 cal., and finally microcalorie (or microcal.) = 0.000001 cal., that is, a millionth of a small calorie. The Greek letter μ has become so thoroughly established in physical terminology that as a short symbol for microcalorie I suggest we use the expression μ-cal.; m-cal. then still remains an expression for 0.001 cal. as Fick had it, but is to be read millicalorie instead of microcalorie.

all before drawing any conclusions it is mandatory that we subject the data for each kind to the same critical examination and to the same final treatment as has been done above for smooth muscle.

In the first place it may be asked whether the data on cross-striped muscle require a correction for vertical displacement of the thermopile coil. The answer is at once, no, for all cases where the contractions were rigidly isometric. But even for the less rigidly isometric twitches, wherever they occur, and in tetanic contractions of two seconds or less, the whole duration of the muscle cycle is too short (in no case more than five seconds) for the displaced coil to experience a disturbing change of temperature. These cases also then may be excluded from further consideration. In cases of fully isotonic contractions, however, the coil displacement is so great that, given a stationary medium filling the muscle chamber, the temperature change is likely to create a considerable thermoelectric effect even in the rapidly acting gastrocnemius and sartorius frog muscles.

As for the H/T values found for Limulus heart I am convinced that these contain no error for vertical displacement of the thermopile coil, for in one case no lever was attached to the heart, the tension (T) having been gotten from the observed pressure changes instead, thus no vertical coil displacement could have taken place; this is for the heart whose H/T value is 11.5×10^{-5}. In the other case a tension lever was attached to the heart but the thermopile was suspended with its coils parallel to the verticle, and was clasped by the tubular heart in such a way that the movement, if any took place, must have been quite negligible.

In taking up the data on terrapin heart attention should be called to the fact, in passing, that the finding for the auricle of this animal is of special interest because the wall is made up largely of smooth muscle fibers. The ventricles, especially, in contracting are allowed to move the thermopile coil vertically more than was the case with the skeletal muscle in the isometric contractions. The masses of the heart muscles, however, varied so much among themselves, and from that of the smooth muscle, that a quite reliable correction factor for the coil displacement can only be had by repeating the experiments and observing the actual thermoelectric effect with the dead or quiescent organ still in place on the thermopile. Making use of the correction factor determined for the smooth muscle in the extreme and median cases of the terrapin ventricles I find this factor varies between three and eight per cent of the observed temperature rise. This error lies well within the limits of error for the whole experimental procedure. Applying the correction to the observed data (the observed tension remaining the same) however, reduces the H/T ratio to 9.3×10^{-5} calorie, a number that more nearly approximates that for skeletal and smooth muscle. (See also Addenda, 1.)

At this point it is in order to mention a criticism that has been directed lately against my work on terrapin ventricle by Fischer (1926). This is not the place to answer this criticism fully, but I wish to say now that my methods and experiments were not strictly used and repeated as that author says he did. In the first place, in my principal studies I used only the split ventricle of the terrapin; this makes a double lobed preparation, the two parts of which are made to straddle a flat coil thermopile as does a pair of sartorius or gastrocnemius muscles. Fischer did not split the ventricle in any case, and in no case did the preparation snugly hug the warm junctions of his thermopile. The method for splitting the ventricle will be found described in my papers of 1917 and 1922. In the second place my muscle chamber arrangement and its temperature regulation were not quite so simple as may be inferred from the incomplete descrip-

TABLE 7

Results on terrapin ventricles corrected for coil displacement, and the final heat production per second of the cardiac cycle

DATE OF EXPERIMENT	ACTUAL MUSCLE SHORTENING (D)	TOTAL TIME OF (M)	OBSERVED MAXIMUM TENSION TOTAL	TIME OF GALVANOMETRIC RESPONSE t'	OBSERVED TEMPERATURE RISE, TOTAL DEG. C × 10^5	CORRECTED TEMPERATURE RISE OF MUSCLE DEG. C × 10^5	WEIGHT OF MUSCLE (W)	$\frac{H}{T}$ CAL. × 10^5	$\frac{H}{TM}$ CAL. × 10^6	$\frac{H}{TM\sqrt{W}}$ CAL. × 10^6
	mm.	*sec.*	*grams*	*sec.*			*grams*			
2/26/21	0.58	34	28.8	26.9	75	69	1.4	2.8	0.83	0.59
5/25/21	0.42	142	28.0	77.2	157	145	2.0	8.5	0.60	0.30
6/ 5/21	0.52	67	38.0	39.5	214	206	3.7	16.5	2.46	0.67
Average....	0.506							9.3	1.29	0.52

tion contained in my earlier papers. It is hoped that the completer description contained in the more recent papers especially in this one and the one by Gemmill (1926) will remove this misunderstanding. From what has been set forth above it is now clear that the error in the results on terrapin hearts is due to vertical displacement of thermopile coil surrounded by a stationary medium. The correction thereof has just been engaging our attention in the foregoing paragraph. To this we shall now return.

If one glances at the H/T values for the ventricle as shown in table 7 one notes that they vary greatly, the extremes (and these are the extremes of the entire series of hearts) being as far apart as 2.8 and 16.5, or nearly 400 per cent. This great divergence would suggest that there is still a large uncontrolled error contained in the results on terrapin hearts.

But that this is not the case was pointed out in my paper on Limulus heart (1926, p. 176), where it was shown that the value of H/T for terrapin ventricle increases with the weight of the muscle and that by dividing the ratio for each ventricle by the weight of the muscle one obtains figures that are much more nearly constant. The reason given for this was the probable increased accumulation of metabolites with increase of weight (volume) of the muscle, a condition that was an inevitable concomitant of the experimental method. But to compare finally the results of the experiments on the ventricle muscle with those on the smooth muscle we shall have first to introduce the time factor (M), and for this I take the whole duration of the cardiac cycle. By doing this, as will be seen in table 7, the divergence is greatly reduced. But even so the mean value of H/TM, 1.29×10^{-6} calorie, is still nearly twice that for the smooth muscle. Upon dividing the ratios for the individual hearts by their corresponding weights, to correct for the disturbing metabolite factor, the divergence is still further reduced and the mean value of the ratio, H/TMW, becomes 0.52 microcalorie. (But see also Addenda, 1.)

The result for the smooth muscle when also corrected for the metabolite factor, the weight being 1.21 gram, gives a value for the H/TMW ratio of 0.53 microcalorie.

The considerations contained in the foregoing three sections may be summed up as follows. At the end of section 5, I stated that in comparing the total heat of the smooth muscle with that of the cross-striped muscles we were comparing two very different things, the heat production of smooth muscle that was tetanized, or at least thrown into prolonged tonus contraction, and cross-striped muscle that was made to contract with a single twitch. In order to put the two things upon a more common basis I then proposed to bring into the equation the total time of the whole muscle action, including the actual time of recovery, and finally the weight of the tissues to correct for an unknown variable probably metabolic in character. Upon doing this a final unit of total heat production is obtained expressing the amount of heat produced per gram weight of muscle per gram weight of tension exerted per second of the whole time of the muscle action and under a uniform (metabolic ?) condition. This standard unit is expressed by the ratio H/TMW, and appears to have the same value for both terrapin ventricle (cross-striped muscle beating spontaneously, and therefore in single twitches), and for terrapin's cardia sphincter (smooth muscle contracting in response to faradisation of its preganglionic nerve fibers). The value of this ratio is about one-half of one microcalorie (0.5×10^{-6} calorie).

8. Summary. The foregoing pages include the following matter:

a. A brief statement of results, method and critique of earlier work on heat production in smooth muscle.

b. A discussion of sources of experimental error;—heat from glandular tissue in the stomach ring; electric currents in the thermopile due to earth induction, and thermoelectric currents due to changes of temperature caused by the vertical displacement of the thermopile in a stationary medium; finally a consideration of possible error from the drifting level of the galvanometer spot during the resting periods of the muscle.

c. Experiments designed to measure the thermoelectric effect of displacing the thermopile coil vertically in the muscle chamber, and the determination of a correction factor for the same to be applied to the observed temperature change accompanying the muscle's contraction.

d. A detailed description of a crucial experiment with the cardia sphincter of a terrapin's stomach when stimulated through its attached motor nerve. This includes a description of the isolation of the preparation, of the muscle chamber and the method used for maintaining constant temperature therein, of the thermopile and the galvanometer used and the method employed of applying the thermopile to the muscle, and of the method of treating the results.

e. A discussion of total and initial heats follows, and the total heat of the smooth muscle preparation when tetanized through its preganglionic nerve fibers is compared with that of skeletal muscle in the single twitch.

f. The heat production per second of the whole time duration of the smooth muscle response is then determined and compared with that in muscle of the cross-striped varieties when reduced to the same standard of measure.

g. The data on cross-striped muscle are reëxamined in the light of this standard of measure, that is, the heat production per gram muscle per gram tension per second of muscle action, H/TMW, and it is shown that its value is nearly the same for all the muscles observed.

CONCLUSION

Physiologists have been accustomed to think of smooth muscle as working more efficiently than muscle of the cross-striped varieties. The foregoing study does not include chemical observations yet the results obtained from the observation of heat production when reduced to a common standard of measure, including the time factor of the whole muscle action, gives no support to the idea that smooth muscle functions with less heat production than do muscles of the cross-striped varieties.

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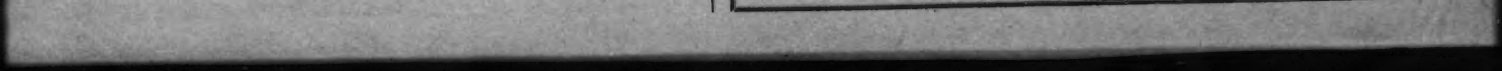
ADDENDA

1. Since this paper has gone to press experiments have been done on two terrapin ventricles in which correction for ventrical displacement of thermopile coil was made according to the method here described for smooth muscle. In these new cases the ratios for $H/T \times 10^5$ turns out to have the values 18.4 and 9.8, the ratios for $H/TW \times 10^5$ the values of 8.0 and 6.7 respectively. But that the H/TMW ratio for ventricle will not always be as small as found for the three ventricles considered in table 7, and for the smooth muscle, appears in the fact that in these two new cases its value is 2.3 and 3.0 microcalories respectively.

2. Terrapin is a common name used in America referring to certain fresh water chelonians and is *not* the opossum (*didelphys virginiana*) as a recent reviewer (L'annee Biol., 1926, p. 439) assumed.



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